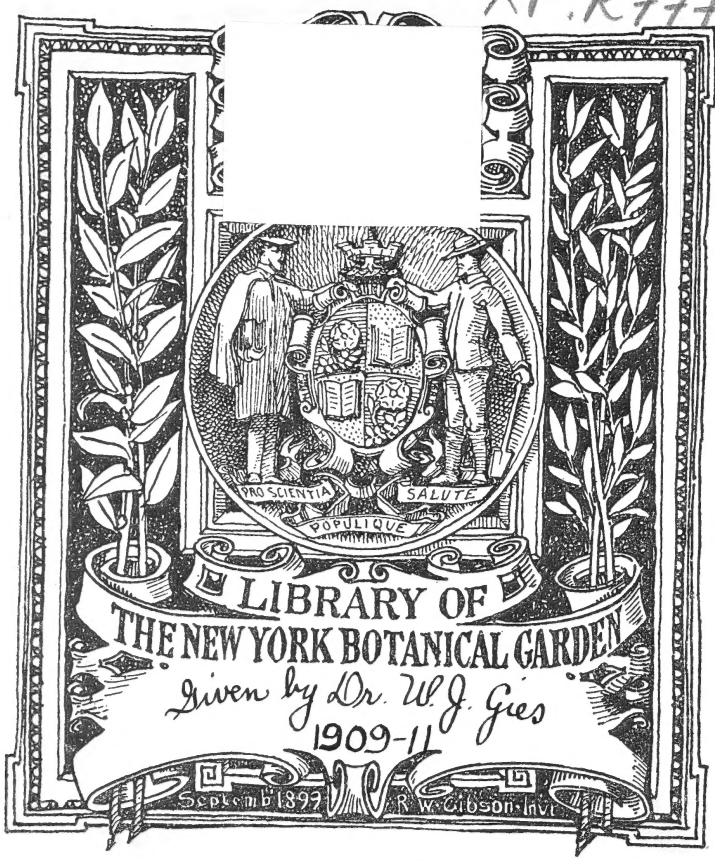
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CONTENTS

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FORTIETH MEETING

COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY

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CONTENTS.

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SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Fortieth meeting.

College of Physicians and Surgeons, Columbia University. October 19, 1910.

1 (526)

How could increase in permeability to electrolytes allow the development of the egg?

By **J. F. McCLENDON.**

[*From the Histological Laboratory of Cornell Medical College and the Laboratories of the Carnegie Institution at Tortugas, Fla., and the U. S. Bureau of Fisheries at Woods Hole, Mass.*]

I found that the electric conductivity of the sea urchin's egg increased about one fourth on fertilization or when made to develop parthenogenetically with sea water containing acetic acid.

Galeotti[1] observed changes in electric conductivity of animal tissues and explained them by the formation and dissociation of ion-proteid compounds. However he observed that the freezing point sometimes did not change appreciably and never changed in proportion to changes in conductivity. Therefore it seems more probable that the changes in conductivity were due to changes in permeability of membranes.

It seems probable, from observations on the volume of the egg, that its osmotic pressure does not appreciably change, even momentarily, on fertilization. For this and other reasons[2] I conclude that the increased electric conductivity is due to increased permeability of the plasma membrane.

[1]Zeit. f. Biol., neue folge, xxv, 289, xxvii, 65.

[2]Dynamics of Cell Division, ii, *Am. Jour. Physiol.*, in press.

Since the egg changes in volume on change in concentration of the electrolytes, as well as some non-electrolytes, in the surrounding medium, it must be less permeable to electrolytes than to water, so by electrolysis one should be able to determine whether it is equally permeable to anions and kations. If an electric current be passed through the egg it begins to disintegrate first at the anode. Loeb and Budgett[1] found that the anodal disintegration of infusoria resembled the dissolution that occurs in alkali more than the coagulation in acid, and concluded that it was caused by the accumulation of kations, forming alkalis, on the outside of the animal. I found that coagulation is not the first effect of all acids. When acetic acid is passed under the cover glass of an infusorian preparation, those individuals first affected die so quickly that the process cannot be analyzed, but those specimens receiving the acid very gradually first undergo dissolution of their membranes and the protoplasm flows out, coagulating only after it reaches acid of greater concentration.

The sea urchin egg undergoes cytolysis in sea water containing acetic acid (as well as in alkalis). On passing a current through sea water in which eggs are placed the cytoplasm disintegrating at the anode is not alkaline to neutral red or to the egg pigment as it should be if the disintegration were due to alkalis massed on the outside of the egg. Furthermore, much less current is required for anodal disintegration of eggs in isotonic sugar solution than in sea water, although in the latter case more kations would be thrown against the anode end of the egg. Therefore it is concluded that the anodal disintegration of the egg is due to confined anions, and indicates relative impermeability to these anions. Probably the anions affect the protoplasm without dissociating water, and some of them may be hydroxyl ions. The anions may change the permeability of the plasma membrane and the swelling of the protoplasm occurs as an effect of the resulting diffusion of undetermined substances.

That the anodal disintegration is due to confined anions is an *a priori* conclusion if the plasma membrane be less permeable than the cytoplasm in general, to ions, since the anions migrating in the interior would be stopped by the membrane but the kations

[1] *Arch. f. d. ges. Physiol.*, lxv, 518 : but see *ibid.*, cxvi, 193.

in the sea water would be free to move around the egg. Höber[1] has shown that the plasma membrane of the erythrocyte is less permeable than the interior, to ions, and this is also probably true of the egg.

In sugar solution unfertilized eggs showed anodal disintegration with less current than did fertilized eggs, indicating that the latter were more permeable to anions, or that the electrolytes had already diffused out, in either case showing an increased permeability to electrolytes.

In a molecular solution of dextrose (approximately isomotic with sea water) fertilized eggs were plasmolysed more rapidly than unfertilized eggs, indicating that the latter were less permeable to the salts exerting the internal osmotic pressure.

To sum up, the increased electric conductivity and liability to plasmolysis with molecular sugar solution and the decreased liability to anodal disintegration, of the fertilized egg, indicate that it is more permeable than the unfertilized egg, to electrolytes. How could this increase in permeability to electrolytes be related to the development of the egg?

Loeb has shown that oxygen and hydroxyl ions in the medium are necessary for development. But eggs will not develop in sea water (which contains oxygen and OH ions) unless some change is induced. Probably this change allows oxygen and hydroxyl ions to reach such a concentration *within* the egg that rapid oxidation may take place. The accumulation of carbon dioxide within the unfertilized egg lowers the concentration of hydroxyl ions. Increased permeability to electrolytes, including carbonic acid, would allow the escape of the latter and the rise in concentration of hydroxyl ions.

The increase in permeability probably causes the increased elimination of carbon dioxide and catalase, and the increased absorption of oxygen, which have been observed by Lyon, Warburg, Loeb and others.

[1] *Arch. f. d. ges. Physiol.*, cxxx, 237.

2 (527)

An inquiry into the nature of the changes in non-regenerating animals.

By **A. J. GOLDFARB.**

[*From the College of the City of New York.*]

It was shown in a recent publication,[1] that very diverse groups of animals, that ordinarily regenerate such organs as the head, tail, leg, etc., could not be prevented from so doing by destruction of that part of the nervous system supplying the amputated region and adjoining parts. And vice versa, that the lack of regeneration was not due to the absence of stimuli passing through the central nervous system.

The present studies pertain to the adult frog, selected as an example of an animal heretofore not known to replace a missing organ, such as the leg. A large series of sections of the amputated region of the leg, prepared at intervals of several days to over nine months, were studied and compared with similar sections of the leg of the newt, which regenerates its limb quite readily. Perhaps the most striking observation centers about the changes of the bone. In brief the evidence supports the view that that which retards or prevents the growth of the bony tissues correspondingly retards or prevents the growth of the adjoining tissues. It was found that while ordinarily no regeneration occurs, yet each of the tissues could, under appropriate conditions, replace the parts originally removed.

These conditions are twofold. In the first place, regeneration of the limb seems to be dependent upon the development of the bony tissues. Ordinarily the exceedingly large growth of periosteum and cartilage is succeeded by regressive changes resulting in a "callous" like structure. When this occurs all further growth changes in other tissues thereupon cease. In one particular instance, the periosteum grew so rapidly that the "anlage" of the leg and foot were distinctly differentiated before the degressive changes set in.

[1]A. J. Goldfarb, The Influence of the Nervous System in Regeneration, *Journal Exp. Zool.*, vii, 1909.

Various operations were performed with a view to preventing an early "callous" formation, and inducing a rapid growth of periosteum and thereby permitting the growth of the other tissues. Most of the legs operated in this way grew distinct "buds" or cones or various malformed or not fully developed limbs.

This incomplete or teratologic regeneration seemed to be due to the influence of a second factor, namely the rate and manner of growth of the connective tissue. This tissue is differentiated quite early, grows about and soon completely surrounds the developing embryonic tissues, apparently preventing their further growth.

Experiments are now under way, to control not only the growth of the periosteum but also this connective tissue, so as to permit, if possible, the complete regeneration of the leg.

3 (528)

Does the pituitary body compensate for thyroid insufficiency?

By **SUTHERLAND SIMPSON** and **ANDREW HUNTER.**

From the Department of Physiology and Biochemistry of the Cornell University Medical College, Ithaca, N. Y.]

Exactly one year ago we submitted to this society the results of some experiments performed on sheep with a view to throw light on the question of the vicarious relationship between the thyroid and pituitary glands. Herring and others had found histological appearances in the pituitary of thyreodectomized animals which suggested a compensatory hypertrophy in that gland, and our object was to determine whether iodine appears in the pituitary of animals from which the thyroid gland has been removed.

The operation of total thyroid extirpation was performed on ten sheep, and the pituitaries removed at death were found to contain no iodine, but we felt at the time that our results were not conclusive, and for three reasons—*first*, five of our sheep died early (six to thirty-two days) from a parasitic infection quite unassociated with the operation; *second*, the available amount of dried pituitary from the ten sheep (1.02 gram) was only sufficient

for a single analysis, and *third*, we had no sure evidence that iodine was being ingested in the food.

In order to be in a position to give a more decisive answer to the question, we have since repeated our experiments on a much larger scale. We removed the thyroid glands completely from eighteen lambs (seven to eight months old) and twelve adult sheep. After the operation they were divided into two groups and boarded out at separate farms in the vicinity of Ithaca, N. Y. The lambs (group I) were fed on clover hay, oats, beets, turnips and small quantities of cotton seed meal; the adult sheep (group II) in addition to this received daily, mixed with their food, half a gram of sodium iodide, to ensure that iodine was being ingested.[1]

No marked symptons followed the operation either in group I or group II and they were killed at the end of five to six months. The pituitary glands were removed, desiccated, and examined for iodine by Hunter's method.[2] The amount of dried pituitary obtained from each group was sufficient for two analyses (four in all) and no iodine was found.

In order to give ample time for the elimination of all circulating iodine, no sodium iodide was administered to group II for ten days before their slaughter.

Our experiments therefore prove conclusively, we believe, that no iodine appears in the pituitary of the sheep after complete removal of the thyroid gland, and assuming that the iodine containing body is the physiologically active constituent of the thyroid gland, as it is claimed to be by Reid Hunt[3] and others, it would appear that the pituitary does not compensate for thyroid insufficiency.

[1]In group I in addition to the eighteen lambs from which the thyroids were removed, one normal lamb was kept as a control. When killed along with the others, the amount of iodine found in the thyroid of this lamb (3.33 mg.) was three times as great as the average (1.11 mg.) for the eighteen at the time of thyreodectomy, five to six months previously. This showed that the natural food did contain iodine although none had been mixed with it as in the case of group II.

[2]Hunter, *Jour. Biol. Chemistry*, vii, May, 1910.

[3]Reid Hunt, Public Health and Marine Hosp. Publications, Bulletin 47, Washington.

4 (529)

The cardio-inhibitory fibers in the woodchuck (Marmotta monax).

By **SUTHERLAND SIMPSON** and **H. W. MAYES.**

[*From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.*]

In mammals the trunk of the vagus in the cervical part of its course, where it is most conveniently exposed and stimulated, is composed of afferent and efferent fibers intimately intermixed. Afferent fibers come from the pharynx, œsophagus, stomach and other abdominal viscera, larynx, trachea, bronchi and pulmonary tissue, and heart; and efferent fibers pass to the voluntary muscles of the soft palate, pharynx, larynx, to the non-striped muscle of the œsophagus, stomach and intestine, and of the trachea, bronchi and their divisions in the lungs, and to the heart.

In studying the functions of the afferent and efferent cardiac fibers by division and excitation, these cannot be separated from each other, nor from the afferent and efferent fibers belonging to other organs, except in the case of the rabbit where the depressor nerve containing the afferent cardiac fibers exists as a separate branch which can be isolated and stimulated alone.

In the woodchuck or American marmot (*Marmotta monax*) we find that the cervical part of the vagus consists of two or three distinct fasciculi which can be readily isolated in the living anæsthetised animal without injury, and stimulated individually. On ligaturing and dividing each of these strands, and stimulating the peripheral and central ends, we find that one of them alone contains cardio-inhibitory fibers. Upwards this can be followed as a distinct fasciculus to the superficial origin of the vagus nerve from the medulla oblongata, but in the lower part of the neck it appears to unite with the other bundles to form a common trunk and cannot be easily dissociated. Whether it contains afferent fibers, or efferent fibers to other organs than the heart, at the present moment we are not prepared to say, but if on further investigation we find that it consists of cardio-inhibitory fibers alone, then by tearing out the fasciculus at its point of exit from

the skull, and pursuing its course centrally by means of the method of indirect Wallerian degeneration employed by van Gehuchten, we shall be enabled to locate anatomically the cardio-inhibitory center in the medulla oblongata.

All that we feel justified in saying in the present preliminary communication, however, is that in the woodchuck one of the bundles into which the vagus nerve can be separated in the cervical region contains cardio-inhibitory fibers, while the others do not. This is unique, so far as we know, and may correspond on the efferent side to the case of the depressor nerve in the rabbit on the afferent side.

5 (530)

The relation between bile-secretion and bile-pressure.

By SUTHERLAND SIMPSON.

[*From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.*]

In the course of our work on the pressure of bile secretion in different animals, Herring and the author[1] found that in some cases the rate of bile flow was greater after the pressure was removed than it had been at the beginning of the experiment. The cystic duct having been clamped, a cannula was tied into the common bile duct and connected by means of rubber tubing to a drop recorder which marked the rate of bile flow on a slowly moving drum. A vertical glass tube mounted on a millimeter scale was introduced by means of a T-piece between the bile duct and the drop-recorder, so that by closing the exit to the drop-recorder the pressure of the secretion could be observed in terms of a column of bile.

In the course of some observations which I have since been making on bile pressure in the sheep, using the same method, I have observed on several occasions that after the bile had risen to its maximum height in the manometer, when the clamp was removed from the outflow tube the rate of flow was much greater than it had been before the pressure began to be recorded and that this increased rate of flow was maintained for a considerable time.

[1]*Proceedings of the Royal Society*, B, Vol. 79, 1907, p. 517.

One explanation of this might be that while the pressure was rising in the manometer the liver was being distended with bile and when the pressure, which was the cause of this distension, was removed the elasticity of the liver tissue led mechanically to the increased expulsion of bile. To test the correctness of this explanation, I killed one animal with an overdose of chloroform when the bile pressure had attained its maximum and at the same time released the clamp obstructing the outflow. In this case the increased rate due to the escape of the bile from the gorged liver very quickly diminished and the flow soon stopped entirely. The cause is, therefore, probably not merely mechanical.

To investigate this matter further, the escape of bile was adjusted so that a certain pressure (170 mm. bile) could be maintained in the manometer while the bile-flow was being registered by the drop-recorder, and in two cases—one sheep and one dog—it was found that the rate of bile-flow was greater than when the bile column stood at zero. At this pressure it is certain that a considerable quantity of bile was passing backwards and being adsorbed into the lymphatics so that the flow through the common bile duct did not account for all the bile that was being secreted. It is right to mention, however, that I observed this in only two experiments out of about twenty.

The most obvious explanation of this phenomenon which suggests itself to me is that the passage of bile through the lymphatics into the blood stimulates the liver cells to increased activity so that more bile is actually secreted. This is all the more probable since we know that the intravenous injection of a small quantity of bile or bile salts acts as a powerful cholagogue. In jaundice by obstruction it would appear therefore, that in the initial stages at all events, a kind of vicious circle is established whereby the bile absorbed stimulates the hepatic cells to produce more bile. This increased activity, which has for its object, no doubt, the removal of some obstruction in the bile passages, will at some stage be arrested, and may give place to partial or complete loss of function.

6 (531)

The role of the concentration of hydroxylions in the antagonistic action of K and Ca upon Na.

By **JACQUES LOEB.**

[*From the Rockefeller Institute for Medical Research, New York.*]

It has been proved during the last years that quite generally in animals, plants and bacteria, a poisonous solution of NaCl can be rendered harmless through the additon of small quantities of $CaCl_2$+KCl. A number of years ago I published results of experiments which indicated that the rôle of Ca in these experiments was fundamentally different from that of K. When fertilized eggs of *Fundulus* were put into *m*/2 solution of NaCl which killed the eggs in a comparatively short time it was found that small quantities of $CaCl_2$ or certain other salts with a bivalent metal rendered the solution comparatively harmless; while the same cannot be accomplished through the addition of KCl, LiCl or NH_4Cl to the *m*/2 solutions of NaCl.

Recently I was able to show this difference in the action of Ca and K in a rather striking way. The fertilized eggs of *Strongylocentrotus* were put in the following four solutions: first NaCl; second, Na+K; third, Na+Ca; fourth, Na+Ca+K. The experiments were made with neutral as well as with acid and alkaline solutions. It was found that in the neutral and acid solution the antagonistic effect of K prevailed over that of Ca, which was very slight; while in alkaline solutions the antagonistic effect of Ca was comparatively strong, while that of K was rather slight.

If newly fertilized eggs of the sea urchin are put into a mixture of NaCl+KCl, one finds that if the solution is neutral, the eggs segment rather normally and they may reach the 64 cell stage. If, however, the solution is made faintly alkaline the eggs are cytolized without segmenting.

If, however, the similar experiment is made with a mixture of Na+Ca one finds that the eggs segment better and further when the solution is faintly alkaline than when it is neutral. To give an example: In a neutral mixture of Na+Ca in one experiment only 40 per cent. of the eggs of *Arbacia* segmented and these did not go

beyond the 2 cell stage; while in the alkaline solution all segmented and reached the 8 or even 16 cell stage. It should be added that the eggs of *Arbacia* will develop in a neutral solution of Na+K+Ca to the blastula stage.

I found also that in mixtures of Na+Mg or Na+Sr or Na+Ba more eggs of *Arbacia* segmented and reached a higher stage of segmentation, when the solutions were slightly alkaline than when they were neutral. These experiments seem to indicate that for the fertilized eggs of the sea urchins the antagonistic action of Ca to NaCl makes itself felt mostly in such processes in which a concentration of HO ions higher than 10^{-7} N is required, while the K ions act antagonistically to NaCl through their participation in processes which may take place in neutral or even slightly acid mediums.

7 (532)

Digestion of protein in the stomach and intestine of the dogfish.

By **DONALD D. VAN SLYKE** and **GEORGE F. WHITE.**

[*From the Laboratories of the Rockefeller Institute for Medical Research and the Laboratory of the U. S. Fish Commission at Woods Hole.*]

Dogfish were fed by a tube with chopped, coagulated beef, and killed after 6, 12, 24, 48 and 72 hours. In the contents of the intestine and stomach determinations were made of (*a*) insoluble nitrogen, (*b*) soluble nitrogen, (*c*) soluble nitrogen in amino form,[1] and (*d*) soluble nitrogen in amino form after complete hydrolysis. The ratio $d : c$ indicates the average size of the peptides composing the peptone mixture.

During the first six hours about one half the ingested protein is dissolved and one fourth absorbed. The unabsorbed peptones are, on the average, of pentapeptid complexity. Very little transfer of stomach contents to the intestine occurs.

At the end of 12 hours 30–45 per cent. of the protein in the tract, including both solid and dissolved matter, is found in the intestine. The peptone in the stomach is broken down to the tripeptid stage, that in the intestine slightly farther.

[1]Van Slyke, these *Proceedings*.

After 24 hours 40–70 per cent. of the nitrogen has disappeared, presumably by absorption, and of that left, in both stomach and intestine, 65–85 per cent. is in solution. The peptone in both stomach and intestine is midway between the di- and tripeptid stages. It remains at this stage until absorption is complete, which occurs in 48–72 hours. After 48 hours, in two cases out of three, only 14 per cent. of the ingested nitrogen remained in the tract.

Urea is constantly present in the intestine. It is excreted with the bile, which contained, in the samples analyzed, 1.7 per cent. of urea, in which form over 70 per cent. of the total bile nitrogen is found.

8 (533)

Absorption and excretion of alimentary nitrogen.

By **DONALD D. VAN SLYKE** and **GEORGE F. WHITE.**

[*From the Laboratories of the Rockefeller Institute and the Laboratory of the U. S. Fish Commission at Woods Hole.*]

Beef and different forms of fish flesh were fed to a dog in nitrogenous equilibrium, the daily diet containing 3 grams of nitrogen, in the form of chopped meat, 65 grams of starch, and 26–27 grams of fat. The animal was catheterized 3, 6, 9, 12 and 24 hours after feeding, and the daily feces were separated by addition of lamp black to the diet. The rise in the rate of nitrogen excretion in the urine was taken as an index of the rate of absorption, the excretion of nitrogen in the feces as a measure of the completeness of absorption. The significance of the results is shown by the following figures:

Food.	Boiled Cod.	Fried Cod.	Boiled Beef.	Boiled Trutog.	Boiled Eel.	Boiled Weakfish.	Boiled Mussel.	Boiled Salt-cod.	Boiled Litorina.
Nitrogen in urine during first 9 hrs. after feeding.	1.50	1.36	1.29	1.28	1.24	1.23	1.23	1.07	1.00
Nitrogen absorbed in 24 hrs.	1.98	1.80	2.58	2.55	1.91	2.53	2.40	2.58	2.37
Nitrogen excreted in urine in 24 hours.	2.51	2.48	2.76	2.35	2.20	2.34	2.22	2.29	1.90
Nitrogen retained.	−.53	−.68	−.18	+.20	−.29	+.18	+.18	+.29	+.47

The foods in the table are arranged in rank according to the

relative rapidity with which their proteins were digested, absorbed, and metabolized, as shown by the rate of nitrogen excretion during the height of digestion (figures in top row). From the nitrogen balance (figures in the bottom row) it is evident that the order would be practically reversed if the foods were ranked according to their ability to keep the body in nitrogenous equilibrium. It is apparent that rapid digestion does not necessarily further retention of protein. Presumably there is an optimum rate of digestion, which makes possible the fullest use of its products, and this rate may be exceeded. Rapid digestion can then cause imperfect retention. "Predigestion" has recently been shown by Voit and Zisterer to have this effect.[1]

9 (534)

The cause of cardiac cohypertrophy.

By **HUGH A. STEWART.**

[*From the Laboratory of the Department of Pathology, Columbia University.*]

In a series of twenty experiments in dogs in which cardiac hypertrophy was induced by the production of aortic insufficiency, it was found that all the chambers of the heart were heavier than normal. The largest increase was in the left ventricle, 48 per cent., but there was also found a very marked increase in the auricles which, relatively to their size, was almost as great as in the left ventricle. The cohypertrophy of the auricles has also been observed in man in cases of chronic interstitial nephritis and arteriosclerosis (Hirsch).

It was found experimentally that the increased work of the left ventricle after the production of aortic insufficiency is not associated with a change in venous pressure such as has been assumed to be the direct cause of hypertrophy of the auricles.

Tracings taken of the contractions of the right auricle along with a blood pressure tracing from the right carotid artery showed that the effect of the production of aortic insufficiency is to increase the force of auricular contraction. Similarly the increased work of the left ventricle produced by the compression of the thoracic aorta will also cause increased auricular contractions without any change in venous pressure.

[1] *Z. f. Biol.*, liii, 457.

The experiments would seem to indicate that there exists a coördination between the contractions of the ventricles and the auricles of such a nature that increased systole of the former causes an increased systole of the latter without any apparent change in venous pressure.

To this condition is ascribed the cause of auricular cohypertrophy.

10 (535)

The origin of convulsions and paralysis following the intravenous injections of the hypertonic solution of sodium chloride.

By DON R. JOSEPH and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In our experiments on the comparative toxicity of chlorides in which, among others, we studied the effects of intravenous injections of a molecular solution of sodium chloride in dogs we observed that after a certain quantity of the solution runs in, twitchings of the muscles of the entire body begin which gradually develop into more or less strong convulsions. Later these gradually grow weaker and finally subside completely, at which time also the respiration stops. The heart continues to beat for several minutes longer. This chain of events is ascribed by pharmacologists to the osmotic action of the hypertonic solution and is generally termed salt action. Loeb however demonstrated that sodium chloride exerts on the living tissue a chemical action also. In the paper embodying the above mentioned experiments we made the following two suggestions: (1) that the twitchings and convulsions are perhaps comparable to the twitching of frog muscles which develop when they are immersed in solutions of sodium chloride; the convulsive movements would be then of peripheral origin; (2) that the subsidence of the convulsions and the paralysis might be due to the curare-like action of the sodium chloride, *i. e.*, to the paralysis of the motor nerve endings.

In the present series of experiments we have tested these two hypotheses. The second hypothesis was tested by investigating

the irritability of the peripheral end of the sciatic nerve during the entire course of the experiment. It was found in every experiment that stimulation of the sciatic nerve even at the time of complete paralysis of respiration did not fail to elicit a definite motor response. This disposes of the second hypothesis; the paralysis is surely not due to a curare-like action of the sodium chloride. The first hypothesis we have tested in various ways. It is known that the twitchings of frog muscles in sodium chloride subside when calcium is added to the solution. We have therefore tried to introduce at some stage of the experiment solutions of calcium chloride into the circulation. In none of these experiments were the twitchings or the convulsions affected in any way by the addition of the calcium solution. Furthermore when the sciatic nerve was cut on one side the muscles innervated by this nerve did not take part in the twitching and convulsions. This fact was more strikingly demonstrated in experiments in which the lower half of the spinal cord was removed. In these cases the contrast between the convulsing upper half and the paralyzed lower half of the animal body was striking indeed.

It is therefore evident that the convulsions and paralysis caused by hypertonic solutions of sodium chloride have their origin neither in the muscles nor in the peripheral nerves; they originate in the spinal cord.

We may append here the brief remark that the convulsions under discussion can be greatly inhibited by intravenous injection of a non-fatal dose of potassium cyanide. We were stimulated to this latter observation by the known experiments of Loeb on the action of cyanide upon the fertilized and non-fertilized sea-urchin eggs.

11 (536)

Simultaneous graphic registration of gastric and duodenal peristalsis in rabbits; a demonstration.

By DON R. JOSEPH and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

The graphic registration of gastric or intestinal peristalsis is usually obtained from an animal with an opened abdomen while

in a saline bath and more or less under the influence of an anesthetic. This method hardly reproduces the normal condition as the act of laparotomy reduces and modifies greatly the peristalsis. In some instances the movements of the stomach have been studied from a gastric or œsophageal fistula and in the rabbit the movements can be studied graphically, as it has been discovered by Auer, in the perfectly normal and unanesthetized animal.

In the April meeting of last year we presented some tracings showing the effect of magnesium upon gastric and duodenal peristalsis. At the present time we wish to explain more fully the method we have used and to demonstrate the act of obtaining the peristaltic tracing. This rabbit was operated four days ago. The movements of the stomach and of the duodenum are transmitted to the kymograph by means of catheters which carry at one end small balloons of thin rubber. The balloon end of one catheter is introduced and secured in the pyloric part of the stomach and that of the other in the descending part of the duodenum through openings made directly in each of these parts. By means of a manometer and a rubber bulb which are interpolated between the catheter and a Marey's tambour the little balloons in the gut can be distended. When not studying the peristalsis the soft catheters are secured by means of a bandage around the animal. The peristalsis is usually obtained when the animal is in a normal crouching position. Soon after the operation there is very little peristalsis to be noticed. In the first few days the peristalsis improves from day to day in character, intensity and regularity. Three to five days after the operation the peristalsis seems to assume a normal character, which, however, we shall not discuss in the present communication. We wish to state, however, that in our earlier experiments in which the duodenal balloon also was introduced through the stomach we lost most of the rabbits within 24 or 36 hours after the operation. The administration of a small dose of physostigmin soon after the operation improved our results greatly. At present we introduce the catheter into the duodenum directly through an opening in the wall of the latter. Our results are now very satisfactory.

12 (537)

The method of inheritance of two sex-limited characters in the same animal.

By **T. H. MORGAN.**

[*From the Department of Zoölogy, Columbia University.*]

At the last meeting of the society I reported the occurrence of a white-eyed mutant in a pedigree culture of the fly *Drosophila ampelophila*. The mutant bred to his red-eyed sisters produced 1,237 red-eyed (male and female) and 3 white-eyed (male) individuals. This sporadic occurrence within the strain is due to further sporting in the eggs of certain females. The same strain has continued to produce white-eyed mutants and these are always of the male sex.

This white-eyed condition has shown itself to be sex limited in its inheritance, *i. e.*, in certain combinations the character is transferred to one sex only. For instance—If a white-eyed male is bred to a red-eyed individual all of the offspring are red-eyed (males and females). These inbred produce red-eyed males and females and white-eyed males. The reds are to the whites as 3 to 1. Thus half of the grandsons inherit the new character but none of the granddaughters.

This result can be explained by means of a very simple hypothesis. If R=red eyes; W=white eyes, and X=the sex factor (one X being a male and XX a female); then the red female fly will be RXRX and her eggs RX and RX; the white male will be WXW and his two classes of spermatozoa WX and W. When crossed the following combinations result.

RX − RX
WX − W
RXWX − RXW
red female red male

When these individuals are paired the outcome is shown by the following formulæ:

RX − WX
RX − W
RXRX = red female
RXWX = red female
RXW = red male
WXW = white male

Another mutant that shows itself to be sex limited in inheritance has appeared in these same cultures. A few males appeared with wings half the normal length.

One of these short-winged males bred to a normal female of another stock produced long-winged males and females. These inbred have produced 5,856 long-winged males and females, and 85 short-winged males (no short-winged females). Here again the character is transmitted from the grandfather to some of his grandsons but to none of his 3,000 granddaughters.

The next question to determine is the relation in inheritance of these two characters, both sex limited. At present I can give only a provisional statement, since the experiment is still in progress.

In the experiment last described the short-winged male had red eyes. He was paired to a long-winged white-eyed female. The offspring consisted of red-eyed females and white-eyed males (the normal result for this combination). All had long wings. These were inbred and produced in the second generation red-eyed, long-winged males and females, white-eyed long-winged males and females, white-eyed short-winged males and red-eyed short-winged males.

In other words, neither white eyes nor red eyes were limited to the short-winged males.

The results are difficult to explain fully owing to the great deficiency of the short-winged forms, due possibly to incompatibility. Let R = red eyes; W = white eyes; L = long wing; S = short wing; X = the sex factor; then: RXSWS = the red-eyed short-winged male, and WXLWXL = the long-winged white female. Their germ cells will be

WXL – WXL
RXS – WS
WXLRSX – WXLWS
red ♀ long white ♂ long

The germ cells of these individuals, and the results of their combination should be:

WXL – WXS – RXL – RXS, red ♀
WXL – WS white ♂
WXLWXL = white ♀ long wings
WXLWXS = " " " "
WXLRXL = red " " "

WXLRXS = red	♀	long	wings
WSWXL = white	♂	"	"
WSWXS = "	"	short	"
WSRXL = red	"	long	"
WSRXS = "	"	short	"

NOTE ADDED NOVEMBER 20.

The converse cross, viz., short-winged, white males by long-winged, red females gives also in the second generation besides long-winged, red- or white-eyed, males and females, short-winged red- or white-eyed *males*.

Heterozygous white females (WXLWXS) by short-winged red males gives long-winged red females, short-winged red females, long-winged white males and short-winged white males.

13 (538)

The glycogenolytic strength of blood serum from the pancreatico-duodenal vein and from the femoral artery, and of lymph from the thoracic duct, as affected by stimulation of the great splanchnic nerve.

By **J. J. R. MACLEOD** and **R. G. PEARCE.**

[*From the Department of Physiology, Western Reserve University Cleveland, Ohio.*]

The distribution of diastatic ferment (glycogenase?) in the animal body would lead one to conclude that its site of production is in the pancreas. Thus:

1. Extracts of this gland possess a glycogenolytic activity which is enormously greater than that of extracts of any other gland, or of blood serum.

2. Blood serum contains the next largest amount of glycogenase.[1]

These considerations prompted us to see whether blood from the pancreatico-duodenal vein is stronger in glycogenase than blood from the femoral or carotid arteries. They were found to be the same. We have recently repeated the observations with the modification that some of the samples of blood were collected during

[1]Macleod & Pearce, *Amer. Jour. of Physiology*, 1910, xxv, p. 255; *cf.* also Wohlgemuth and Benzur, *Biochemische Zeitschrift*, 1909, xci, p. 460.

stimulation of the great splanchnic nerve. In these experiments we have also determined the glycogenase in lymph, collected from the thoracic duct. The experiments were conducted in the usual manner[1] and in most cases controls with 0.5 per cent. soluble starch were run. These gave similar results to those in which glycogen

Experiment No.	Nature and Amount of Fluid Used.	Experimental Condition.	Glycogen (Dextrose) in Incubation Flasks.			Remarks.
			At Start.	After Incubation.	Disappeared.	
III.	Fem. art.	Before stimulating gt. spl. nerve.	0.087	—	—	Incubated 3 (?) hrs. 1 c.c. serum. Starch test gave dextrines first with *.
	Do.	After 15 min. stim.	"	0.014	0.073	
	Pan. duo. vein.	Before stim.	"	0.019	0.068	
	Do.*	After 15 min. stim.	"	0.011	0.076	
	Lymph.	Before stim.	"	0.020	0.067	
	Do.	After 15 min. stim.	"	0.012	0.075	
IV.	Fem. art.	Before stimulating gt. spl. nerve.	0.185	0.077	0.108	Incubated 3 hours. 1 c.c. serum. Starch test gave dextrines in 60 min. with all.
	Do.	During 1 hour stim.	"	0.077	0.108	
	Do.	30 min. after stim. off.	"	0.082	0.103	
	Pan. duo. vein.	Before stim.	"	0.082	0.103	
	Do.	During 1 hour stim.	"	0.082	0.103	
	Do.	30 min. after stim. off.	"	0.080	0.105	
VI.	Fem. art.	Before stimulating gt. spl. nerve.	0.141	0.067	0.074	Incubated 4½ hrs. 1 c.c. serum in each case. Starch test gave dextrines first with *.
	Do.	During 30 min. stim.	"	0.040	0.101	
	Do.	45 min. after stim. off.	"	0.062	0.079	
	Pan. duo. vein.	Before stimulating.	"	0.060	0.081	
	Do.	During 30 min. stim.	"	0.059	0.082	
	Do.	45 min. after stim. off.	"	0.051	0.090	
	Lymph.	Before stim.	"	0.053	0.088	2 c.c. serum incubated 3½ hours.
	Do.*	During 30 min. stim.	"	0.026	0.115	
	Do.	45 min. after stim. off.	"	0.040	0.101	
VIII.	Fem. art.*	Before stim.	0.146	0.081	0.065	Incubated 1 hour. 1 c.c. serum. Starch test gave dextrines first with * * *.
	*	During 20 min. stim.	"	0.081	0.065	
	*	80 min. after stim. off.	"	0.070	0.076	
	Lymph.	Before stim.	"	0.095	0.051	
		During 20 min. stim.	"	0.099	0.047	
		60 min. after stim. off.	"	0.101	0.045	
IX.	Fem. art.	Before stim.	0.140	0.065	0.075	Incubated ½ hr. 5 c.c. serum.
		During 80 min. stim.	"	0.058	0.082	
		60 min. after stim. off.	"	0.047	0.093	
	Pan. duo. vein.	Before stim.	"	.—	.—	
		During 80 min. stim.	"	0.058	0.082	
		60 min. after stim. off.	"	0.060	0.080	

was employed. The incubation periods varied from 30 minutes to 4 hours and 30 minutes, and the amounts of serum or lymph varied

[1]Macleod & Pearce, *loc. cit.*

from 1 c.c. to 5 c.c. By thus varying the conditions, chances of error are greatly eliminated. Controls were run in all the experiments except in some of the estimations of experiments 3 and 8 in which there was not sufficient serum or lymph for this purpose. Table I gives the results of these experiments.

Consideration of Results.—In experiments IV and VIII there was no evidence of increase of glycogenase during stimulation of the splanchnic nerve. There was a slight increase in the ferment contained in the femoral blood after the stimulation in experiments VIII and IX, but, since this was 60–80 minutes after the stimulation had been removed, the increase cannot be due to the stimulation. In experiment III there was a slight increase both in the pancreatic vein and in the lymph immediately after the stimulation. In experiment VI there was an increase in the lymph and in the femoral artery blood during the stimulation. The only experiment, therefore, in which splanchnic stimulation certainly caused a rise in glycogenase was No. VI. The increase occurred primarily in the lymph and secondarily in the systemic blood. It fell off in both after the stimulation was removed. We are not in a position at present to determine whether this result is of any importance in the metabolism of carbohydrates in the animal body, but we do not believe that it has any relationship to the increased glycogenolysis which occurs in the liver as a result of stimulation of the splanchnic nerve. Did it have this relationship we should expect the increase in glycogenase to occur in the blood of the pancreatico-duodenal vein rather than in the lymph.

14 (539)

The influence of an inoculation with tumor material of experimentally decreased virulence upon the result of a second inoculation with tumor material of experimentally decreased virulence.

By ELLEN P. CORSON WHITE and LEO LOEB.

[*From the Laboratory of Experimental Pathology of the University of Pennsylvania and from the Department of Pathology of the Barnard Free Skin and Cancer Hospital, St. Louis.*]

Eight years ago one of us has shown that through the influence of chemical and physical factors it is possible to decrease the energy of growth of tumor-cells without killing the cells.[1] While a certain degree of injury kills the cells, a graded decrease in the action of the external agency leads to corresponding gradations in the decrease in the energy of growth of the cells. Thus it is possible to obtain tumor material which is still alive, but which gives rise to a very insignificant growth in many instances, and the initial growth of which is in many cases followed by a spontaneous retrogression. At that time we considered the possibility that such tumor material of experimentally decreased virulence might serve as a vaccine which might provide a certain protection against a second injection with more virulent material.

We tested therefore the influence of a first injection of such material upon the growth of a subsequent inoculation with tumor material. In the majority of cases both inoculations were separated by an interval of 10–20 days.

In order to obtain a more sensitive reagent indicating the presence of even a slight immunity, we used as material for the second (or sometimes third) test-injection not virulent tumor pieces, but again tumor material of experimentally (through heating) decreased energy of growth. Our results were as follows:

1. If the tumor material of experimentally decreased virulence did not give rise to tumor formation, no immunity was noticeable, even if the mice had received two or three preparatory injections

[1] *Virchow's Archiv*, Bd. 172, 1903, p. 395.

at various intervals. In a number of cases the last inoculation gave rise to even larger tumors than in the control mice which had not received any preparatory inoculation. In cases in which mice were injected with a suspension of finely divided tumor material of (through heating) diminished virulence, the same lack of immunity was observed on subsequent inoculation with tumor pieces of decreased virulence. The number of mice which we treated with a preparatory injection of fine tumor suspension is however as yet relatively small and these experiments will therefore have to be continued.

2. If the preparatory inoculation with material of decreased virulence led to a temporary tumor growth, which latter was followed by a spontaneous retrogression an immunity against a subsequent inoculation with virulent tumor material was obtained in many cases. This observation is in accordance with the findings of other investigators. This immunity is however by no means absolute and it will be necessary to determine in further experiments upon what factors such gradations in acquired resistance depend.

3. In many cases mice in which a tumor has begun to grow after the first inoculation of tumor material of decreased virulence, can be successfully inoculated a second time with material of decreased virulence, and in some experiments the tumors derived from the second transplanted piece grow as well in mice in which the first preparatory inoculation had given rise to tumor growth as in such mice in which the first pieces had not given origin to tumor growth, and in such animals two tumors are growing simultaneously; but in the majority of cases we find that only one tumor grows after two successive inoculations with tumor material of decreased virulence. If the preparatory inoculation had been followed by tumor growth, the second inoculation gave frequently a negative result and vice versa. And especially if the tumor resulting from the first inoculation is growing very actively, a second inoculation with material of decreased virulence is in the large majority of cases not followed by tumor growth. This observation would therefore point to the conclusion that in a certain number of cases the growth of a tumor protects to some extent against a subsequent inoculation with material of de-

creased virulence. But in as much as in a number of experiments such an excluding action of a first inoculation was not noticeable, we are not yet ready to state positively that such an immunizing effect of a growing tumor exists. We are however inclined to believe that a number of variable factors (especially a variable energy of growth of the first and second tumors) are present and that such factors complicate the results. In further experiments we shall endeavor to analyze such variable factors.

15 (540)

Supplementary report on attempts to immunize against tuberculosis.

By **J. P. ATKINSON** and **C. B. FITZPATRICK.**

[*From the Department of Health, City of New York.*]

We have sought to employ the resistance acquired by or embodied in the serum of the healthy animal in its life struggle against a deadly infection with tuberculosis.

The depression reaction produced by injections of tuberculous serum into animals sensitised with tuberculin, a description of which we have already published has led us to this investigation.

After employing a number of methods for utilizing the serum of healthy animals which had been infected with tuberculosis, we are duplicating one which seems to have given promising results.

Method 1—Begun December 2, 1909. The result of this experiment was reported at the meeting of the American Association for the Advancement of Science, December 27, 1909.

One rabbit was given a sensitising dose of 1 c.c. tuberculin subcutaneously and beginning two days later this rabbit received the following:

Dec. 4,	1 c.c	Tuberculous rabbit serum.
" 6,	"	" " "
" 8,	"	" " "
" 10,	2 c.c.	" " "
" 14,	"	" " "
" 16,	"	" " "
" 18,	"	" " "

A rest of ten days was given the experimental rabbit which was

then given a fatal dose of a culture of bovine tuberculosis intravenously. Two control rabbits were given an equal amount of the same culture. The controls lived 30 days during which time they lost weight and presented a typical tubercular appearance. The autopsy showed marked and characteristic lesions of tuberculosis.

The experimental rabbit lived 39 days and kept in good condition up to one week before its death and then lost weight rapidly. The autopsy showed the same marked and characteristic tubercular lesions as the controls.

The fact of the long-continued good physical condition of the experimental rabbit encouraged us to repeat this experiment with some changes.

January 8, 1910. Two rabbits were sensitised with ¼ c.c. each of crude tuberculin which was followed on the twelfth inst., by injections of 3 c.c. each of tuberculous rabbit serum. This procedure was repeated three times and the serum alone given six times.

The following is the table of injections, all subcutaneous.

January 8, each ¼ c.c. crude tuberculin.
" 12, 3 " tuberculous rabbit serum.
" 14, ¼ " crude tuberculin.
" 17, 3 " tuberculous rabbit serum.
" 19, ¼ " crude tuberculin.
" 21, 5 " tuberculous rabbit serum.

One rabbit died on January 21. The other one received on

January 24, ¼ c.c. crude tuberculin.
" 26, 5 " tuberculous rabbit serum.
" 28, 2½ " " " "
" 30, 2½ " " " "
February 3, 3 c.c. tuberculous rabbit serum.
" 5, 5 " " " "
" 7, 5 " " " "

A rest of 11 days was given the surviving rabbit, and then on February 18, 1910, it was given a fatal dose of bovine tuberculosis intravenously. Two control rabbits were given the same amount of the bovine tuberculosis culture.

One control died April 5, the second control died July 11; both these rabbits showed marked and characteristic tubercular lesions. The experimental rabbit died July 17, from pneumonia.

The autopsy showed that the lungs, liver, kidneys, suprarenal capsule, spleen and glands were free from tubercular infection.

Both controls lost weight consistently until death.

The experimental rabbit held its weight up to a week before its death when it lost weight very rapidly.

This rabbit appears to have been successfully protected by injections of tuberculin and tuberculous rabbit serum from a fatal dose of a culture of bovine tuberculosis. We are now duplicating this experiment with two experimental rabbits and three controls.

On June 25, 1910, two variations of the previous methods were used. Two rabbits were used for each experiment. Variation 1 consisted in alternate injections of crude tuberculin and tuberculous rabbit serum as follows:

June 25, each rabbit ¼ c.c. tuberculin.
" 27, " " 2 " tuberculous rabbit serum.
" 28, " " ¼ " tuberculin.
" 29, " " 2 " tuberculous rabbit serum.
" 30, " " ¼ " tuberculin.
July 1, " " 2 " tuberculous rabbit serum.
" 2, " " ¼ " tuberculin.
" 4, " " 2 " tuberculous rabbit serum.
" 5, " " ¼ " tuberculin.
" 6, " " 2 " tuberculous rabbit serum.
" 7, " " ¼ " tuberculin.
" 8, " " 2 " tuberculous rabbit serum.

Variation 2 consisted in alternate injections of tuberculous rabbit serum and that portion of the tubercle bacillus split by alcoholic potash which is insoluble in the alcohol. Vaughan claims that this portion of the tubercle bacillus sensitises but does not poison. This is true of the preparation we used as will be described further on.

The following table gives the injections:

June 25, each rabbit 1/10 gm. insoluble residue.
" 27, " " 2 c.c. tuberculous rabbit serum.
" 28, " " 1/10 gm. insoluble residue.
" 29, " " 2 c.c. tuberculous rabbit serum.
" 30, " " 1/10 gm. insoluble residue.
July 1, " " 2 c.c. tuberculous rabbit serum.
" 2, " " 1/10 gm. insoluble residue.
" 4, " " 2 c.c. tuberculous rabbit serum.
" 5, " " 1/10 gm. insoluble residue.
" 6, " " 2 c.c. tuberculous rabbit serum.
" 7, " " 1/10 gm. insoluble residue.
" 8, " " 2 c.c. tuberculous rabbit serum.

(One of these rabbits was bitten and died from a resulting infection, August 1.)

These four rabbits with three controls were each given an intravenous injection of a lethal dose of bovine tuberculosis on July 22, 1910, after an interval of 12 days since the last injection of tuberculous rabbit serum.

These rabbits died and showed marked and characteristic lesions upon autopsy except the rabbit which died on August 1, as the result of a bite and one that died August 22, and was not autopsied. This animal, however, showed physical signs of tuberculosis before death.

The following table shows the time of death of the experimental rabbits and the controls.

Controls 1—died August 8.
" 2— " " 12.
" 3— " " 15.

Experimental rabbits—Tuberculin and Tuberculous rabbit serum.
1—died August 12.
2[1]— " " 22.

Experimental rabbits—Tuberculous rabbit serum and insoluble residue.
1—died from infected bite August 1.
2— " August 10.

In order to test the properties of the products obtained from the treatment of the tubercle bacilli according to the directions of Dr. Vaughan the following tests were made.

Some of the soluble portion injected subcutaneously into a guinea pig produced the following symptoms: nervousness, chilliness, weakness in the hind quarters, convulsions and death in about 45 minutes.

Injections of the alcoholic insoluble portion did not cause symptoms of any sort.

A small quantity of this insoluble portion was injected into each of three guinea pigs. After three weeks two of these pigs were injected intraperitoneally with an emulsion of tubercle bacilli, from which the split products had been prepared, with the following results.

Pig No. 1. After 30 minutes, restlessness followed by weakness in the hind legs; violent convulsions followed by extreme fatigue. These convulsions continued during the day and the pig died finally in a convulsion 6 hours after the injection.

[1]No. 2 had lost a great deal in weight and was undoubtedly tuberculous.

The autopsy showed undissolved bodies of tubercle bacilli spread over the omentum.

Pig No. 2. Some restlessness but no other sign that it was affected by the injection. Two days later this pig was seized with a convulsion and died.

The autopsy showed undissolved bodies of tubercle bacilli not spread over the omentum but collected together in small groups. The edge of the spleen was covered with small abscesses which did not show tubercle bacilli.

The serum of rabbits treated according to experiment two (2) acquired a clumping property for the tubercle bacilli. This was shown by each of the two rabbits that are now duplicating the successful experiment No. 2.

An emulsion of bovine tuberculosis was mixed with clear serum drawn from these animals six days after the last injection.

A mixture of this serum from each rabbit and a uniform suspension of tubercle bacilli after standing overnight at room temperature was found to cause well defined clumping. The control in normal sodium chloride solution was not clumped.

A knowledge of substances which counteract the toxic effects of the infecting organism is of great value in attempts at immunization.

The fact that "tuberculin" the toxic extract of the tubercle bacillus is an arterial blood depressor immediately suggests that the extract of the suprarenal gland might antagonize it. We have carried out experiments of this nature and find that according to the amount of tuberculin and suprarenal extract in the mixture one can produce a depression or rise at will.

The depressor substance in tuberculin then can be measured by suprarenal extract of known strength.

If the depressor substance in tuberculin is a necessary constituent for tuberculin as a diagnostic and therapeutic agent, then we have an easy and simple method of measuring its strength.

We have injected tuberculin into dogs from which we have removed one and both suprarenals. The results bear out the theory, but have brought to light so much that is new and interesting that we will reserve the discussion for a future paper.

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Members elected at the fortieth meeting:

John Howland, R. A. Lambert, Ernst Sachs, H. B. Williams.

Dates of the next two regular meetings:

December 21, 1910. February 15, 1911.

PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

FORTY-FIRST MEETING

THE ROCKEFELLER INSTITUTE FOR
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CONTENTS.

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SCIENTIFIC PROCEEDINGS.

Abstracts of the Communications.

Forty-first meeting.

The Rockefeller Institute for Medical Research. December 21, 1910.

16 (541)

Retention of normal polarity in centrifuged stems of *Tubularia*.

By **Max W. Morse.**

[*From the Harpswell Laboratory and the Biological Laboratories of Trinity College, Hartford, Connecticut.*]

Stems of the hydroid, *Tubularia crocea*, were cut from the colony, which had been taken directly from the sea. The hydranths were removed by cutting them from the stems immediately below their attachment. A small branch from the stem was left intact to serve for orientation, and the hydranth which belonged to this branch was cut off.

These stems were placed in specially prepared tubes fitted to a hematocrit centrifuge, operated by hand. Rotation was made at varying speeds and for varying intervals of time ranging from a minimum speed of rotation of about two hundred and forty per minute to a maximum of about six hundred rotations per minute. A greater speed than this resulted in forcing the contents of the perisarc tube out at the distal end. The periods of time of rotation varied from a minimum at highest speed of one minute to a period of maximum operation at medium number of rotations per minute (approximately three hundred, the centrifuge being about one-in-four, but there was no speedometer available to accurately measure the number of rotations at the higher speeds), for one half hour.

The behavior of *Tubularia* stems under all of these conditions was uniform and unvarying. When regeneration occurred, which was true in 100 per cent. of the cases under observation, as the stems lay horizontally in finger-bowls, the hydranth appeared at

the originally *distal* end of the stem, regardless as to whether the distal or proximal end of the stem had been directed centrifugally or centripetally in the experiment and regardless of the fact, that as in the higher speeds, the contents of the perisarc tube were compressed into the end of this tube which was directed centrifugally in rotation. This compression varied with the centrifugal force involved, but at the higher speeds, the contents which filled the tube for a distance of four centimeters, would be compressed, in the experiment, into a space measuring about five millimeters. When regeneration took place, the red pigment which marks the future hydranth pole could be seen collecting in the compressed protoplasm and gradually it migrated up the tube of perisarc until it reached the end of this tube, whereupon the tentacles and other parts of the normal hydranth appeared.

That the red pigment has no rôle as a "formative stuff" has already been shown by Morgan according to evidence derived from another method of approaching the point and the present set of observations appears to show that if any stratification of "formative stuffs" occurs in the normal stem of the hydroid, whereby hydranth forming stuffs and stolon forming material are relegated to their respective ends of the stem, these stuffs are not responsive to the action of centrifugal force in the degrees used in the experiments or else they become rearranged when the centrifugal action has ceased. The generalization may be made that polarity in *Tubularia crocea* cannot be altered by the action of centrifugal force, in shifting "organbildende Bezirke" from one end of the hydroid stem to the other.

17 (542)

Creatin and creatinine metabolism during convalescence after typhoid fever.

By **NELLIS B. FOSTER.**

[*From the Wards of the New York Hospital and the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons, New York.*]

On account of the apparently intimate connection between "muscle efficiency" and the output of creatinine in human urine

it seemed of some moment to investigate the creatin and creatinine excretion during convalescence from acute febrile disease. Patients convalescent from typhoid fever were selected because during the course of this disease there is considerable loss of muscle tissue, and on that account if creatin or creatinine take part in the synthetic processes of muscle regeneration, their utilization for this purpose might be more easily detected than in conditions where the metabolism is less active. Young adults and children were used exclusively, since it is reasonable to suppose that in such subjects anabolism occurs at its height.

The diet in all cases consisted of milk, eggs and cereals exclusively.

The following tables are compiled from the records of analyses which were made during two weeks of convalescence in each of these cases.

From these tables it may be noted that when creatinine is ingested under the conditions of these experiments a large part of the material is recovered in the urine, but there is always a loss which remains to be accounted for. When creatin is fed it can not be recovered as creatin in the urine, unless the patient is on a diet very rich in protein and the amounts of administered creatin are large. A slight increase in creatinine excretion which at times follows the ingestion of creatin is too small to warrant any conclusion.

The occasional presence of creatin in the urine of these patients is not to be explained by any observation made clinically. All of the patients were without fever during the convalescence and relapses occurred in no instance. The presence of even traces of creatin in urine under these circumstances is of interest in its bearing upon the earlier observations of Munk.[1]

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[1] *Deutsch. Klinik*, 1862, p. 300.

Case I. (Weight 50 K.)

Day of Observation.	Amount of Urine, c.c.	Total N, gm.	Creatinine, gm.	Creatin, gm.	Remarks. Amount Fed.[1]
1	1,820	9.12	0.910		
2	1,450	8.96	0.838		
3	1,150	8.26	0.810 (average 0.853 gm.)		
4	1,400	7.09	0.994		0.47 gm. creatinine
5	1,930	8.84	0.965		
6	1,480	9.89	0.915		(recovered 0.3 gm.)

Case II. (Weight 28.6 K.)

1	850	4.70	0.456		
2	1,430	9.35	0.448		
3	1,770	12.72	0.478		
4	1,490	7.99	0.440 (average 0.455 gm.)		
5	1,730	12.27	1.073		0.94 gm. creatinine
6	1,910	11.52	0.439		(recovered 0.62 gm.)

Case III. (Weight 60 K.)

1	1,150	10.99	0.831	0.005	
2	2,020	10.72	0.997 (average 0.914 gm.)	0.004	
3	1,100+	9.74+	0.781+	0.006	
4	1,720	11.15	1.734	none	1.639 gm. creatinine.
5	1,710	10.12	0.968	0.058	(recovered 0.82 gm.)

Case IV. (Weight 28.1 K.)

1	1,000	6.63	0.250	0.105	
2	1,150	6.53	0.220	0.125	
3	1,010	8.63	0.287 (average 0.252 gm.)	0.063	
4	1,000	8.09	0.310	0.074	.3 gm. creatin.

Case V. (Weight 45.9 K.)

1	1,305	3.27	0.741	——	
2	1,120	9.01	0.605	——	
3	1,790	4.00	0.895	0.004	
4	1,410	10.41	0.635 (average 0.719 gm.)	0.036	
5	1,105	8.34	0.500	0.046	0.66 gm. creatin.
6	1,220	9.70	0.588	0.010	

Case VI. (Weight 59.5 K.)

1	1,260	9.88	0.788	——	
2	1,650	12.56	0.765 (average 0.776 gm.)	——	
3	1,700	10.01	0.935	0.018	1.31 gm. creatin.
4	1,720	10.41	0.989	0.017	
5	1,430	11.29	0.667	——	

Case VII. (Weight 40 K.)

1	1,300	32.57	0.438	0.065	
2	580	25.23	0.451	0.109	
3	1,370	34.94	0.444 (average 0.444 gm.)	0.107	
4	1,410	32.99	0.524	1.411	3 gm. creatin.
5	840	26.46	0.616	0.338	

[1]The amounts of creatin and creatinine given were computed from the weight of substance on the basis of quantitative estimation by Folin's method.

18 (543)

Creatinine and creatin metabolism in dogs with Eck fistula.

By **NELLIS B. FOSTER** and **HENRY L. FISHER.**

[*From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons, New York.*]

In connection with certain studies of hepatic function the creatin and creatinine metabolism were investigated in two dogs with Eck fistula. This subject was studied last year by London and Boljarski.[1] They found that the administration of creatinine did not increase urinary creatinine, and that the feeding of creatin caused no increase in creatin excretion but was followed by a slight increase in eliminated creatinine.

The diet given our dogs consisted of unsweetened condensed milk, cracker meal, lard and water. Dog I took food with no apparent hunger and would at no time eat the prescribed amount, some days taking scarcely any. To this, in part is to be assigned the rapid loss in weight. Dog II took his food well and maintained constant weight.[2]

A study of the tables shows that ingestion of creatinine increased the creatinine output in the urine. The excretion of this substance is not quantitative in every instance, though at times this is approximated. There was no clear cut effect upon creatin excretion after giving creatinine. Following the ingestion of creatin there is no corresponding rise in creatin excretion; a slight rise in the creatinine output is suggestive but not convincing. Both creatinine and creatin appear to act as diuretics and the increase in nitrogen excretion on those days is possibly due to a washing out of excretory substances rather than to a conversion of creatin or creatinine into some other substance previous to excretion. The disproportion between the urinary nitrogen and the amount of substance administered suggests this explanation:

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[1] *Zeit. f. Physiol. Chem.*, 1909, lxii, 465.

[2] Both dogs were examined carefully at autopsy to ascertain if a collateral circulation had rendered the Eck fistula useless for our purpose. In Dog I no such condition could be detected, but in Dog II a minute aberrant branch of the splenic vein was found which in some slight degree might have diminished the effects of the operative anastomosis.

Dog I. Eck Fistula.

Date, 1910.	Body Weight, Kilos.	Amount of Urine, c.c.	Total N, gram.	Creatinine, gram.	Creatin, gram.	Amount Fed.
April 11	11.85	625	3.79	0.209	None	
12	11.81	410	2.41	.167	.040	
13	11.45	690	4.50	.364	.084	2.33 gm. creatin.
14	11.60	330	3.77	.251	.046	
15	11.50	340	2.81	.219	.027	
16	11.50	300	2.47	.135	.015	
17	11.30	420	5.46	.399	.032	
18	11.20	490	2.79	.172	.014	
19	11.35	840	6.29	.756	.043	0.60 gm. creatinine.
20	11.05	240	2.89	.166	.017	
21	10.80	420	4.99	.318	.067	1.2 gm. creatin.
22	10.68	550	3.57	.234	.063	
23	10.12	780	7.64	.694	.208	0.35 gm. creatinine.
24	10.07	425	3.62	.187	.161	

Dog II.

July 2	10.67	170	4.76	0.418	0.017	
3	10.35	130	3.06	.346	.078	
4	10.85	240	6.92	.381	.004	
5	10.65	160	5.87	.350	.041	1.11 gm. creatin.
6	10.70	115	2.58	.253	.002	
7	10.60	100	2.94	.288	.080	1.0 gm. creatin.
8	10.65	430	9.99	.507	.009	0.3 gm. creatinine.
9	10.60	180	4.69	.460	.000	
10	10.78	135	2.56	.360	.008	
11	10.72	160	4.96	.373	.076	
12	10.68	320	3.14	.326	.014	
13	10.71	175	3.14	.378	.004	
14	10.70	190	3.04	.363	.017	
15	10.76	420	3.31	.340	.008	
16	10.72	355	4.12	.387	.161	1.07 gm. creatin.
17	10.65	230	2.77	.324	.000	
18	10.68	160	3.89	.301	.010	
19	10.67	300	3.03	.486	.011	0.5 gm. creatinine.

[Compare the data for the 13th with the 21st day and those for the 19th with 23d of Dog. I.[1]] The creatin used in these experiments gave no reactions for creatinine. We are indebted to Dr. S. R. Benedict for creatinine made from this creatin. The amounts were computed from weights of substance on the basis of quantitative estimation by the Folin method.

[1]We are indebted to Drs. E. H. Poole and H. H. Janeway for operative procedures.

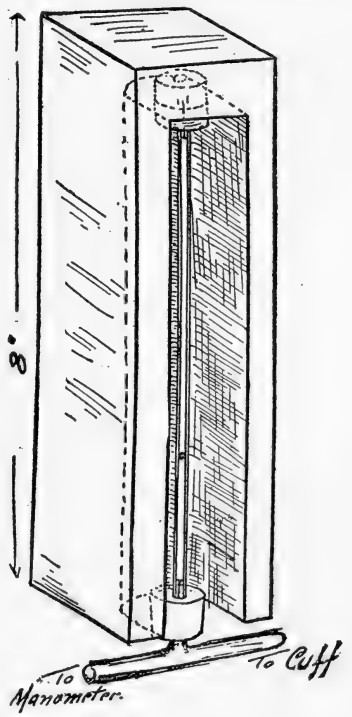
19 (544)

A blood pressure apparatus with pith-ball attachment indicating diastolic pressure.

By **NATHANAEL FEDDE.**

[*From the Laboratory of Physiology of the Cornell University Medical College.*]

Of the many factors governing blood pressure, the most important, namely the force of the heart beat, receives least attention. A knowledge of the systolic pressure is of value in warning of certain dangers, but gives no clue as to the amount of work the heart does. In order to estimate this we must know the difference between the pressure during the heart beats and that in the intervals. The maximum pressure is easily ascertained by any one of the numerous sphygmomanometers. Only a few have means, in any way satisfactory, for determining diastolic pressure, and these are prohibitive in price and of tremendous bulk. To overcome these obstacles we have devised a small instrument that can be attached to any sphygmomanometer. This consists in an air chamber communicating by means of a glass tube containing a pith-ball with the tube leading from cuff to manometer. Any increase of pressure in the cuff is equalized in the chamber by a rush of air passing the pith-ball and moving it. With a steady slow exhaust of air the ball moves exactly as the point of Erlanger's lever, but without the fling. An average pulse throws Erlanger's lever about 3 cm., while the pith-ball flies about three times that distance. The movements of the ball are interpreted just as the movements of the lever in Erlanger's instrument. That point of pressure is read at which the oscillations begin to fall off from the maximum.



20 (545)

Some desirable results following water drinking with meals.

By **P. B. HAWK.**

[*From the Laboratory of Physiological Chemistry of the University of Illinois.*]

In the continuation of our studies on the influence of water drinking at meal time, data have recently been collected as to the influence of this factor upon some of the activities of the gastro-intestinal tract. Particular attention has been given to the stimulation of gastric secretion, the activity of the pancreatic function, and to the course of intestinal putrefaction. As regards the stimulation of gastric secretion it has been found that the stimulation is directly proportional to the volume of water ingested. The activity of the pancreatic function measured by the fecal amylase (Wohlgemuth's method) was found to be increased during the water period. At the same time intestinal putrefaction, as measured by the indican content of the urine (Ellinger's method) was decreased. Absorption was also facilitated and the excretion of fecal bacteria lowered when large volumes of water were ingested at meal time.

21 (546)

Metabolism after hypophysectomy.

By **C. G. L. WOLF** and **E. SACHS.**

[*From the Department of Chemistry, Cornell University Medical College, New York City.*]

Three out of a series of respiration experiments on sixteen dogs, in which a part or the whole of the hypophysis was removed were reported.

They represented: (1) Extirpation of part of the anterior lobe and all of the posterior lobe. (2) Removal of the anterior lobe, leaving the pars intermedia and the posterior lobe. (3) Complete hypophysectomy.

The amount of gland destruction was determined by serial sections of the brain after the autopsy of the animals. Control experiments were made to determine the effect of the operative

procedures without removal of the gland. There was no effect on the carbon dioxide output.

In case 1 there was a distinct lowering of the carbon dioxide output, and a low level was established which persisted for sixteen days after the operation. At the end of this time the animal, which had previously been retaining nitrogen, was again in nitrogen equilibrium. On autopsy, an increase in fat and atrophy of the ovaries was observed. The weight of the animal had increased.

In case 2 there was a lowered carbon dioxide output, although marked emaciation followed the operation.

Case 3 lived but 48 hours after the operation. A respiration experiment was performed 24 hours after hypophysectomy. There was a markedly decreased carbon dioxide output, and an unusually low nitrogen output for the same period.

The apparatus used in these experiments was a modified Pettenkofer-Voit. The periods used for the determinations were six hours.

22 (547)

The influence of calcium and of sodium in M/10 solution upon the conductivity in nerve trunks.

By **DON R. JOSEPH** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In a previous communication before this Society[1] we reported that calcium chloride in an *M*/10 solution is capable of reducing or completely abolishing the direct and indirect irritability of frog muscles. The reduction or abolition is reversible; sodium chloride restores rapidly the lost irritability. It was further found that the primary action of calcium does not affect both forms of irritability in an equal manner; in a number of cases, especially under certain conditions of temperature and season, a comparatively small amount of calcium solution abolished completely the indirect irritability (from the nerve) while the direct muscle irritability still persisted in nearly its original intensity. From these experiments we concluded, among other things, that calcium af-

[1] Joseph and Meltzer, PROCEED. OF THE SOC. FOR EXPER. BIOL. AND MEDICINE, vol. vi, p. 104, 1909.

fects the motor nerve endings more readily than the muscle tissue; in other words, *calcium, like sodium, potassium, and magnesium exerts a curare-like action upon the motor nerve endings.* However, there was one link missing in the evidence in favor of the conclusion mentioned. The abolition of the indirect irritability might be due to the action of calcium upon the nerve trunk, and not upon the nerve ending. We have therefore studied the action of calcium chloride upon the nerve trunk in a short series of experiments. The results of this study form the subject of our present communication.

In our former studies the calcium solution was administered to the muscle by intravascular perfusion. For our present study bathing of the nerve in the solution was the method which had to be employed. A very small cup made of a section of glass tubing was filled with this solution, into which a loop of the sciatic nerve was immersed and kept down by a small pledget of absorbent cotton saturated with the same solution. The brim of the cup was slightly covered with vaseline to prevent the overflow of the solution. The section of the sciatic nerve between the cup and the gastrocnemius muscle was covered with a pledget of cotton saturated with Ringer. The same was the case with the lumbar plexus which was used for stimulation and kept on an appropriate electrode. The graphic registration of the contractions of the gastrocnemius muscle were obtained in the usual manner. The drum was turned by hand at arbitrary intervals. The stimulations were accomplished by single induction shocks (break) which at the beginning of the test gave a maximal contraction. Every few minutes the effect of a stimulation of the lumbar plexus was tested, comparing it sometimes with the effect of a similar stimulation of the part of the sciatic nerve peripheral to the cup.

In every experiment both legs were used at the same time: one for testing the effect of an $M/10$ calcium chloride solution and the other to study the action of an $M/10$ sodium chloride solution. Only the effect upon the conductivity was studied; the loop was never taken out of the cup to test also the effect of the solutions upon the irritability.

Ten experiments were made, nine of which gave the following uniform results which we shall state very briefly.

Primary action of calcium chloride.—In every experiment a time came when the conductivity of the nerve trunk became finally abolished; stimulation of the lumbar plexus gave no reaction, while stimulation of the distal part of the sciatic nerve brought out a good response. This, however, occurred only after prolonged bathing. Ninety minutes was the shortest period; in some cases it took 150 minutes and longer before all response from the lumbar plexus disappeared.

This result bears out our original conclusion. In our former experiments the indirect irritability disappeared after a few minutes exposure to the action of the calcium chloride. This could not have been due to the action of the calcium solution upon the nerve trunk since calcium is able to produce abolition of conductivity only after hours of bathing.

Reversible; restored by sodium chloride.—In every experiment the vanished conductivity came back after replacing the calcium solution by an $M/10$ solution of sodium chloride. The conductivity returned in a comparatively short time, probably in less than 15 minutes. After recovery, the lumbar plexus responded in a manner similar to that of the distal part of the sciatic nerve, which after several hours of exposure to the abnormal surroundings usually lost somewhat of its original irritability.

Primary action of sodium chloride.—It has been established already by Locke, and by Overton, that physiological salt solution does not affect the conductivity of the nerve trunk. Our experiments simply confirm these statements. After many hours of bathing of the nerve in an $M/10$ sodium chloride solution the lumbar plexus lost indeed some of its original irritability; but the loss was not greater than that of the distal section of the sciatic nerve which was kept covered throughout the experiment with cotton saturated in Ringer. We ought to add that the temperature of the laboratory during the period in which the experiments were carried out (November) was by no means low.

23 (548)

Observations on the nitrogen content of the succus entericus.

By **HERMAN O. MOSENTHAL, M.D.**

[*From the Laboratory of Biological Chemistry and from the Department of Medicine of Columbia University, at the College of Physicians and Surgeons, New York.*]

The succus entericus in these experiments was obtained by means of a "Thiry" fistula in dogs. The nitrogen content and the quantity of the succus entericus secreted by the entire small intestine of each dog in twenty-four hours was estimated by multiplying the amounts collected from the fistula in a given period by two factors: first, by the figure necessary to bring the number of hours up to twenty-four, and second, by the figure required to compensate for the length of the dog's small intestine, as determined at autopsy.

Summarizing the protocols, the following average figures are obtained:

	Number of Observations.	Weight of Dog, kilos.	N Intake, gms.	N of Succus Entericus, Calculated for 24 Hours, gms.	N of Feces, gms.	N of Urine, gms.	N of Succus Entericus as Per Cent. of:		
							N Intake.	N Feces.	N Urine.
Dog 1	3	13.9	8.8	3.1	0.8[1]	8.0[1]	35	383	39
2	5	11.7	6.7	2.4	0.6[1]	5.0	34	393	47
3	15	7.0	7.4	1.5	0.7	5.6	21	225	27
4	14	7.1	4.3	1.9	0.3	3.2	43	607	59

Conclusions.—Nitrogen to the amount of about 35 per cent. of the food nitrogen of a mixed diet, is daily secreted in the succus entericus in dogs. Of this quantity, an amount equal to about 10 per cent. of the food nitrogen is excreted in the feces and an amount equal to about 25 per cent. of the food nitrogen is reabsorbed. The amount of reabsorbed nitrogen-containing material is considerably larger if the bile and pancreatic secretion are included.

The metabolic significance of this reabsorption can only be surmised; that it is probably of great importance is indicated by the fact that in the experiments presented, so large an amount of nitrogen, *i. e.*, equal to approximately 25 per cent. of the nitrogenous part of the food, is concerned.

[1] The value is estimated; was not determined.

24 (549)

The relation of the adrenals to tuberculin poisoning.

By **JAMES P. ATKINSON** and **CHAS. B. FITZPATRICK.**

[*From the Chemical and Research Laboratories. Department of Health, City of New York.*]

A paper which we read before this society in the spring of 1910 showed that "old" tuberculin or the filtered fluid from a culture of *B. tuberculosis* caused a marked arterial depression in the dog when injected into the femoral vein. This same tuberculin if heated above 105° lost this power of depression.

We have recently found that commercial adrenalin preparations and saline extracts of the experimental dog's freshly removed adrenal gland prevented this depression when mixed with tuberculin and the mixture injected intravenously.

Samples of tuberculin, which caused marked drops in the blood pressure, when mixed with a sufficient amount of an emulsion of the dog's own adrenals and injected intravenously, caused no fall in the blood pressure, thus showing an antagonistic action between tuberculin and the adrenals. The intravenous injection of samples of tuberculin containing the active depressor substance, after the adrenals had been removed, caused a drop with a more gradual recovery to the previous pressure level, than when the adrenals had been left intact. The intravenous injection into the dog with both adrenals removed of an emulsion of its own adrenals caused a return of the blood pressure to and above the normal level.

Further light is apparently thrown on the nature of the poisons of the tubercle bacillus as found in tuberculin by the following observations.

A. Four tuberculous guinea pigs were injected with a mixture of .5 c.c. of "P. D. & Co. Adrenaline Chloride 1–1000." Three of the pigs died within 18 hours and one within 36 hours. One control, a tuberculous pig, receiving .5 c.c. tuberculin without adrenalin died within 18 hours. The other control, a normal guinea pig, receiving .5 c.c. of the adrenalin chloride "P. D. & Co. 1–1000" did not apparently suffer any harmful results or show any noticeable toxic symptoms.

Four tuberculous guinea pigs injected with a mixture of .5 c.c.

tuberculin and 2 c.c. of a 1/100 dilution of "P. D. & Co. Adrenalin Chloride 1–1000" died within 18 hours. The control, a guinea pig, infected with *B. tuberculosis*, with enlarged glands, was injected with 2 c.c. of the same diluted adrenalin solution and did not show any toxic symptoms and lived.

B. .5 c.c. of the same tuberculin used in the previous experiments heated from 106° C. to 110° C. for 1 hour killed a tuberculous guinea pig within 18 hours.

We have studied the nature of tuberculin poisoning further by injecting tuberculin into dogs after one and both adrenals have been removed. The characteristic kymograph reaction of a tuberculin injection into normal dogs is a quick fall followed by an almost equally quick rise in pressure to the previous level.

The injection of a depressing dose of tuberculin into the dogs with the adrenals removed caused a marked drop with a much delayed response in the return to the previous pressure level.

Repeated successive depressing doses, varying from .5 c.c. to 5 c.c. for each dose, of samples of tuberculin injected intravenously into several large dogs which had had both adrenals removed did not cause a drop to the base line or immediate death, but the dogs lived from 2½ to 3 hours after the removal of the adrenals.

The average life of a dog after both adrenals have been removed is about 40 hours. Little or no ether was required after both adrenals had been removed to keep the dogs anesthetized. Clotting, which after the intravenous injections of samples of tuberculin in several doses, varying from .5 c.c. to 5 c.c. for each dose, had always seriously interfered with our work with the kymograph, was entirely absent after the adrenals had been removed even when tuberculin had been repeatedly injected.

We have repeatedly injected normal dogs in order to sensitize them with 5 c.c. of crude tuberculin without apparent injury or noticeable toxic effects.

These results indicate:

A. That tuberculin is a complex substance consisting of at least two poisons, one a blood pressure depressor destroyed by heat and antagonized by adrenalin; the other a substance characterized by its fatal effects on tuberculous guinea pigs when injected sub-

cutaneously and by its stability, which resists heating to 110° C, and which is not neutralized by adrenalin.

B. It is possible that we have evidence here that the absence or the abnormal diminution of the adrenal secretion permits some of the pathogenetic action of the products and extracts of the tubercle bacillus and that their administration mixed with the whole adrenal or some part of it, in a measure overcomes their deleterious action.

C. It is possible that the neutralizing value of blood mixed with tuberculin or some other appropriate adrenal antagonist might be a valuable index of the functional condition of the adrenal glands.

25 (550)

Comparison of the blood-flow in the hands in a case with lesion of upper motor neurones (birth palsy) and in a case with lesion of lower motor neurones (infantile paralysis).

By **G. N. STEWART.**

[*From the Department of Experimental Medicine, Western Reserve University.*]

The blood flow was calculated from the formula

$$\varphi = \frac{H}{T - T'} \times \frac{1}{S}$$

where φ is the quantity of blood flowing through the hand in the period of observation, H the heat given off to a calorimeter containing the hand, T the temperature of the arterial blood coming to the hand (taken as rectal temperature),[1] T' the temperature of the venous blood leaving the hand (taken as the average temperature of the water in the calorimeter[2]) and S the specific heat of blood. Before being put into the calorimeter the hand was immersed for a sufficient time (usually ten minutes) in a large

[1]Observations since made on the actual temperature of the arterial blood show that in a healthy man the rectal temperature is about half a degree above that of the blood coming to the hand under the conditions of the experiments. The temperature of the arterial blood is arrived at by determining that temperature of the calorimeter at which the hand neither loses nor gains heat.

[2]That this assumption is approximately correct for a certain range of bath temperature has been shown by actual measurement of the temperature of blood issuing from one of the veins of the hand, with suitable precautions to render the loss of heat as small as possible.

bath containing water at the same temperature as that in the calorimeter in order that T' might approximate to the temperature of the calorimeter. All thermometers used were, of course, compared with a standard thermometer. In the cases observed, the two hands were simultaneously immersed in two precisely similar calorimeters. The volume of the hand enclosed in the calorimeter was determined by afterwards immersing the hand up to the proper level in a lipped beaker previously filled with water and measuring the overflow, or by immersing it in a vessel provided with a gauge.

Case 1.—Man æt. 46, weight 160 lbs., height 5 ft. 10 in. Marked contracture of the flexors of left wrist, with spastic paralysis of hand and fingers; also some deformity of left foot. A history was obtained indicating gradual onset of the contractures. At the age of two deformity was noted in the left hand and slight deformity in the left foot. The condition gradually got worse. The general health is good and the man is able to work as a telegraphist. There is some control of the movements of the left hand. He can extend the fingers and flex them but not normally. He uses the hand to a certain extent in his occupation. It is markedly smaller than the right. The pulse at the wrist is quite distinct. The hand feels as warm as the right one. The case was diagnosed as birth palsy by Dr. Henry O. Feiss, to whom I am indebted for the opportunity of studying it and the other case.

PROTOCOL OF EXPERIMENT.

Three liters of water in each calorimeter.

3.39 P.M. Both hands immersed in bath at 27.8°.

3.45 Temperature of bath 27.4°.

3.49 Left hand put into calorimeter A, right into B.
Patient standing and hands hanging down.

	A.	B.
3.51	27.63	27.60
3.52	27.68	27.60
3.53	27.72	27.66
3.54	27.81	27.73
3.55	27.89	27.81
3.56	27.95	27.95
3.57	28.00	28.01
3.58	28.09	28.13
3.59	28.20	28.29
4.00	28.22	28.33
4.01	28.29	28.40

At 4.01 the hands were rapidly withdrawn and the final reading taken after vigorous stirring. Variations in the previous readings due to inadequate stirring or accidental contact of the fingers with the thermometer are thus controlled.

RATE OF COOLING OF CALORIMETERS AFTER WITHDRAWAL OF HANDS.

	A.	B.	Room.
4.18	27.94	28.10	20.1
4.27	27.85	28.00	
4.36	27.77	27.91	19.5

Volume of right hand in calorimeter 370 c.c.

Volume of left hand in calorimeter 330 c.c.

Pulse rate in standing position 84. Rectal temperature 37.4.

Taking the water equivalent of hand and calorimeter together as 300 grm. and the loss of heat by the calorimeter as 0.01° per minute, we get for the left hand H = 2,508 small calories; T = 37.4; T' (average temperature of the calorimeter) = 27.96. Taking the specific heat of blood as 0.9, we get

$$\varphi = \frac{2508}{9.4} \times \frac{10}{9} = 296 \text{ grm. blood}$$

in ten minutes, *i. e.*, 9.0 grm. blood per minute per 100 c.c. of hand.

For right hand φ = 354 grm. blood in ten minutes, *i. e.*, 9.6 grm. blood per minute per 100 c.c. of hand.

Case 2.—A boy 9 years and 4 months old. Normal delivery, no instruments used. At the age of one month the child had convulsions. At 6 months the mother noticed disability in the left arm and left leg. The arm has since become flaccid, and the whole arm is atrophied. The upper arm can only be raised level with the shoulder. Some power of flexion of the forearm at the elbow but hardly any power of rotation. The hand is held pronated and everted toward the ulnar side and the fingers are extended. They are small and cold. There is some power of flexion of the middle, ring and little fingers but very little in the case of the thumb and index finger. Movements at wrist very slight.

Diagnosis: infantile paralysis.

PROTOCOL OF EXPERIMENT.

At 2—11—30″ P.M.	Both hands put in bath at 26.6°.
2.19	Temperature of bath is 26.5°.
At 2.19	Put hands into calorimeters, each containing 2,800 c.c. water. (These were somewhat smaller calorimeters than those employed for case 1.)
	Patient standing.

	Calorimeter containing left hand.	Calorimeter containing right hand.
2.20	26.32	26.40
2.21	26.36	26.45
2.22	26.40	26.55
2.23	26.53	26.70
2.24	26.54	26.80
2.25	26.54	26.85
2.26	26.55	26.95
2.27	26.60	27.05
2.28	26.61	27.10
2.29	26.65	27.20
2.30	26.65	27.30

The last readings were taken after vigorous stirring immediately after removal of the hands from the calorimeters.

COOLING OF CALORIMETERS AFTER WITHDRAWAL OF HANDS.

	Left.	Right.	Room.
2.43	26.60	27.20	23.4

Pulse 95 in standing position. Rectal temperature 37.6°.

Volume of left hand in calorimeter 170 c.c.

Volume of right hand in calorimeter 210 c.c.

The hands being small, portions of the arms were included.

φ = 111 grm. blood in ten minutes for left hand, *i. e.*, 6.5 grm. per minute per 100 c.c. of hand.

φ = 306 grm. blood in ten minutes for right hand, *i. e.*, 14.5 grm. per minute per 100 c.c. of hand.

Conclusion.—In the hand whose lower motor neurones were not involved in the lesion producing the paralytic condition the blood-flow per unit volume of hand substance is scarcely inferior to that in the normal hand.

In the hand a lesion in whose lower motor neurones is responsible for the paralysis, the blood flow per unit volume of hand substance is 2½ times less than in the normal hand. The difference in the amount and condition of the muscular tissue is one important factor in causing the difference in blood flow in the two conditions.

26 (551)

Edema formation in guinea pigs in chronic experimental uranium nephritis.

By **ERNEST C. DICKSON.**

[*From the Pathological Laboratory of Cooper Medical College.*]

In a series of experiments performed on guinea pigs during the past two years, with the purpose of confirming the findings in experimental chronic nephritis which I have previously reported,[1] some interesting observations in edema formation have been made. Twenty-one animals received subcutaneous injections of an aqueous solution of uranium nitrate, as follows: six received numerous injections of 0.5 m.grms. at frequent intervals; eight received several injections of 5 m.grms. at longer and irregular intervals; and seven received one or more injections of 10 to 15 m.grms. Four animals died within two weeks after the first injection, and can be excluded from the chronic nephritis series. The remaining seventeen survived for from three to twenty-three months after the first injection, and all showed kidney lesions of a chronic nature, similar to those which I have previously described.

In Group I, which received the numerous small doses, two

[1] *The Archives of Internal Medicine*, June, 1909, vol. iii, No. 5.

animals which had received 26 and 80 injections, respectively, survived for seven and twenty-three months, and at autopsy showed no edema. Two which had received 47 and 70 injections, respectively, survived for twelve and nineteen months, and at autopsy showed marked ascites, over 30 c.c. of fluid being collected in the latter case. The remaining animal which received 86 injections survived for twenty-three months, and at autopsy showed marked ascites, and double hydrothorax. In all but one case in this series, death occurred in from two to five days after the last injection.

In Group II, four animals which died within four months after the first injection, showed no edema. One which received 10 injections of 5 m.grms. each, during fifteen months, died two days after the last injection, and showed marked subcutaneous edema, but no accumulation of fluid into the serous cavities. This animal increased 50 grms. in weight during the last two days of life. One animal which received eight injections during thirteen months, died five days after the last injection, and showed moderate subcutaneous edema, marked ascites, double hydrothorax, and hydropericardium. Still another which received four injections during eight months, died eleven days after the last injection, having increased 125 grms. in weight during the last five days of life, and at autopsy showed marked subcutaneous edema, and marked ascites, over 120 c.c. of ascitic fluid being collected. The remaining animal of this series received seven injections during fourteen months, died eight days after the last injection, and showed moderate ascites.

In Group III, one animal which received two injections of 10 m.grms. and one which received a single injection of 15 m.grms. died within four months, and showed no edema. One which received 30 m.grms. in three doses, died in sixteen months, eleven days after the last injection, and showed ascites, double hydrothorax, and hydropericardium. The remaining animal received 20 m.grms. in three injections, died in sixteen months, five days after the last injection and showed moderate subcutaneous edema, and moderate ascites.

In the complete series of seventeen animals which survived for longer than three months, seven which died before the eighth

month showed no edema, and of the ten which lived for longer than eight months, nine showed definite, and in some cases, marked edema. In four of the nine cases there was marked subcutaneous edema, which in three cases was associated with effusion into the serous cavities. All of the cases of subcutaneous edema occurred in Groups II and III, where relatively large doses of the drug were administered.

In none of the cases was any attempt made to induce edema formation by forcing water. The animals were fed upon a mixed diet in which was a plenty of greens, and they obtained all their water from the green food. Since death occurred in every case but one within eleven days after the last injection, and during the resulting acute intoxication, it would seem that the edema was a true renal edema, and not due to stasis resulting from a broken down heart. There can be little doubt that the prolonged action of the uranium nitrate upon the blood vascular system had so damaged it, that it was unable to withstand the strain of the plethora which was produced by the inability of the kidney to excrete water during the terminal attack of acute nephritis.

27 (552)

The action of infundibulin upon the mammary secretion.

By **ISAAC OTT** and **JOHN C. SCOTT.**

[*From the Physiological Laboratory, Medico-Chirurgical College of Philadelphia.*]

In the goat we have found in the early nursing period that infundibulin (the active principle of the posterior part of the hypophysis), when injected into the vein of the ear, rapidly and greatly increased the secretion of milk. The nipple had a cannula inserted into it, and a water aspirator produced the suction necessary to empty the udder. The milk before and after the injection was caught in a graduated flask and measured every five minutes. The following experiment will given an idea of the activity of the infundibulin:

GOAT—RIGHT NIPPLE.

2.25 P.M.
2.30 " ..... 4 drops milk.
2.35 " ..... 5 " "
2.40 " ..... 5 " "

2.41 P.M.	5 drops of infundibulin by the vein.				
2.45	"	.....	405	drops	milk.
2.50	"	.....	15	"	"
2.55	"	.....	22	"	"
3.00	"	.....	12	"	"
3.05	"	.....	8	"	"
3.10	"	.....	4	"	"
3.11	"	5 drops of infundibulin by the vein.			
3.15	"	.....	75	drops	milk.
3.20	"	.....	15	"	"
3.25	"	.....	15	"	"
3.30	"	.....	7	"	"
3.35	"	.....	6	"	"
3.40	"	.....	5	"	"
3.45	"	.....	4	"	"

Care was taken to thoroughly empty the udder both by aspiration and by rhythmic external compression of the gland. The intra-venous injection of infundibulin starts the flow in about one minute from the beginning of the injection, and it reaches its height in four minutes, after which it rapidly falls to normal.

28 (553)

The galactagogue action of the thymus and corpus luteum.

By **ISAAC OTT** and **JOHN C. SCOTT.**

[*From the Physiological Laboratory, Medico-Chirurgical College of Philadelphia.*]

In experiments upon the goat with the glands containing internal secretions, we have found the corpus luteum, pineal body and thymus increased the quantity of milk fourfold in five minutes. The ovary minus corpus luteum had no effect. Infundibulin is still the most powerful galactagogue, increasing the secretion of milk one-hundredfold. The amount of butter fat was about the same in the augmented secretion by thymus, corpus luteum, and infundibulin, but occasionally it was increased.

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Dates of the next two regular meetings:

February 15, 1911. April 19, 1911.

PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

FORTY-SECOND MEETING

CORNELL UNIVERSITY MEDICAL COLLEGE

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CONTENTS.

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SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty-second meeting.

Cornell University Medical College. February 15, 1911.

29 (554)

Cell Division and Cell Regeneration. I. Uronychia transfuga.

By **GARY N. CALKINS.**

[*From the department of Zoölogy, Columbia University.*]

1. Although many experimenters have studied the regenerative power of different kinds of protozoa no one has observed the variations in this power at different age periods after division.

2. *Uronychia transfuga* is a marine hypotrichous ciliated protozoon about 100μ in length. It may be cut in any plane with a fine pointed scalpel and the pieces will live for several days, thus showing a hardiness which makes it a good subject for experimentation.

Under conditions of culture in the laboratory (at Roscoff, France) the cell divides about once in 36 hours, the period between divisions being marked by definite changes in size of the cell and in arrangement of the nuclear elements (macronucleus and micronucleus).

3. Experiments to test the regenerative power were made upon cells immediately after division; on cells from 12 to 18 hours after division; on cells immediately before division; and on cells during division. The cells, in all cases, were cut with a scalpel under a microscope, full records of 75 experiments being kept.

4. If cut immediately after division the fragments continue to live for at least three days, but that fragment alone regenerates which contains macronuclear material and the micronucleus. The other fragment contains a variable amount of macronuclear material but no micronucleus. In two of the thirteen successful experiments on cells in this stage, neither fragment regenerated.

In other cases regeneration of the micronucleus-holding fragment was completed in from twelve to eighteen hours.

5. In cells cut from twelve to eighteen hours after division and after full size is attained, the results are similar to those on cells immediately after division. The micronucleus-holding fragment alone regenerates, the other fragment, containing only macronuclear material lives for several days but fails completely to regenerate. This result was obtained in twelve different cases.

6. If cut just prior to division both fragments regenerate within 24 hours, the one fragment containing only macronuclear material, the other, containing both macronucleus and micronucleus. The latter regenerates more rapidly than the former. One cell regenerates without a micronucleus.

7. If cut during the early stages of division three completely regenerated cells result, one of which (the fragment cut off) contains no micronucleus. Division continues in the original plane, the result being one normal cell and one minute cell perfect in all respects save size. Regeneration of the micronucleus-holding cells is more rapid than that of the third fragment. This result was obtained in fourteen cases.

If the cells are cut during the later phases of division the results are similar to those obtained by cutting immediately after division. One fragment regenerates, the other fails. This result was obtained in seven out of eight experiments on cells in this stage, the other one gave regeneration of both fragments.

8. The experiment shows a considerable variation in the power to regenerate in this form; this power is lowest immediately after division, but it gradually increases with age after division until it reaches a maximum immediately before the next division and during the early phases of division.

During division it decreases to a minimum in the later stages and immediately after division.

9. The results might be interpreted by the assumption of a specific substance, possibly enzymatic in nature, which accumulates with age of the cell until a condition analogous to saturation is reached. With the formation of the new cell-organs this substance, it may further be assumed, is exhausted and regeneration

is impossible save with the full complement of cell organs (macro- and micronucleus and cell protoplasm).

10. Experiments in cutting *Paramecium caudatum* were also made. Here regeneration of the cell does not occur save under exceptional conditions which I shall report at length upon later. A frequent result is the formation of monsters with from two to fourteen mouths; an abnormality due to some derangement of the cellular mechanism through the removal of a small portion of the cytoplasm.

30 (555)

Iodine as a skin disinfectant in animal surgery.

By **H. W. MAYES.**

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

In experimental physiology where it is necessary that the animal be allowed to recover after the removal of an organ or the establishment of a lesion, the operative procedure is carried out under full anæsthesia and with aseptic or antispetic precautions as in human surgery. The preliminary disinfection of the skin by scrubbing with soap and water and the subsequent washing with bichloride, carbolic or alcohol, takes considerable time and in most cases must be done after the animal is anæsthetized; besides where the operative field includes the head or face there is always danger of the eyes being accidentally injured by the irritative fluids. Then again, after operation everyone has experienced the difficulty of keeping the dressing properly applied to the wound which must be protected from outside contamination unless the animal be kept in aseptic surroundings—a condition practically impossible in most laboratories. Any method, therefore, which will materially save time and trouble and at the same time not increase the risk is particularly desirable in animal surgery and such a method, I believe, is to be found in the use of iodine as a skin disinfectant.

Iodine was first applied in human surgery about fifty years ago by Bryant[1] and Boinet[2] and recently it has come into vogue

[1] *Brit. Med. Journ.*, 1910, I., p. 1003.

[2] *The Lancet*, 1910, CLXXIX., p. 1888.

again. The results are claimed to be eminently satisfactory but there may be objections to its application to the comparatively sensitive skin of the human subject on account of its irritating qualities which do not obtain in the case of animals.

The method adopted by me, which is a modification of that employed by Grossich, is as follows: Immediately before the animal—*e. g.*, cat or dog—is put into the ether chamber the hair is cut short and the skin shaved dry along the line of the intended incision. Tincture of iodine (U.S.P. 7.5 per cent. in 95 alcohol) is painted on and a little way around the shaved area with a camel hair brush and the anæsthetic is administered. When the animal is fully under (with ether this usually takes ten to fifteen minutes) and placed on the table a second application of the iodine is made a minute or two before the skin is incised, and a third, after the stitches are in, when the operation is finished. No dressing of any kind is applied to the wound but the day following the operation a fourth application of the tincture is made. No further treatment is necessary,

During the last four months many operations have been performed in this laboratory on a variety of animals—rabbits, cats, dogs, sheep, raccoons, opossums, etc., and in every case where the above procedure has been adopted healing has been by first intention and rapid.

31 (556)

The Diagnosis of Abortive Cases of Poliomyelitis by the Demonstration of Specific Antibodies.

By **JOHN F. ANDERSON** and **WADE H. FROST.**

[*U. S. Public Health and Marine Hospital Service, Washington, D. C.*]

The occurrence of abortive cases of poliomyelitis has already been established upon clinical and epidemiological ground. Netter and Levaditi[1] have given the only specific proof of an abortive case of poliomyelitis in a human being by demonstrating, in the serum of such a case, immune bodies capable of neutralizing the

[1] Netter, A., & Levaditi, C., *Compt. Rend. de la Soc. de Biol.*, vol. 68, 1910, pp. 855–857.

virus of poliomyelitis *in vitro*. Their case was a child which suffered a clinically obscure, mild illness about the same time that another child of the same family suffered a frank attack of poliomyelitis.

We have undertaken a similar demonstration in nine suspected abortive cases of poliomyelitis selected from a much larger number observed during an epidemic of acute anterior poliomyelitis in Iowa in the summer of 1910. Special interest is attached to these cases because of the mildness of their symptoms, the frequency of similar cases and their epidemiological relation to cases of frank poliomyelitis.

The sera of these nine cases were tested for immune bodies as follows: One half cubic centimeter of each serum was mixed with an equal volume of a 5 per cent. emulsion (filtered through paper) of fresh spinal cord from a monkey in the acute paralytic stage of poliomyelitis following inoculation with virus (M.G.) kindly furnished by Dr. Simon Flexner, of the Rockefeller Institute. To each mixture was added 0.1 c.c of fresh serum from a normal adult. As controls, we used the serum of a frank case of poliomyelitis and of a normal adult. The mixtures were allowed to stand 1 hour at 27° C. and 20 hours at 15° C. One half cubic centimeter of each mixture was then injected intracerebrally into a monkey (*Macacus rhesus*). The two control monkeys receiving the mixture of normal serum and virus developed typical poliomyelitis on the tenth and twelfth day respectively. Three of the monkeys receiving the serum from suspected abortive cases also developed poliomyelitis.

The monkeys receiving the serum from the other six suspected abortive cases, and from the frank case of poliomyelitis have all remained well (78 days).

We then repeated the test, using the serum of the three suspected abortive cases which had failed to show immune properties in the first experiment; and for controls, using five specimens of normal human serum (three adults and two children). In this series we altered the proportion of serum and virus to the extent that we used a *one per cent.* emulsion of fresh spinal cord, all other conditions remaining the same. In this series poliomyelitis developed in only two of the controls, and in one of the monkeys

receiving the serum of a suspected abortive case of poliomyelitis, indicating presumably a certain degree of germicidal power even in *normal* human serum.

To compare this germicidal property of normal serum with that shown by the sera from the six suspected abortive and two paralytic cases in series 1, we inoculated a third series of monkeys. In this series we tested the three normal sera which had shown neutralizing properties in series 2 using a *5 per cent.* emulsion of virus, thus repeating exactly the conditions of series 1. All three monkeys developed typical poliomyelitis in 10 to 14 days.

The sera of six out of nine (66.7 per cent.) suspected abortive cases of poliomyelitis have shown, when tested against the virus of poliomyelitis, a germicidal property greater than that shown by any one of six normal sera similarly tested. We interpret this as establishing, to a reasonable certainty, the diagnosis of poliomyelitis in these six cases, and strongly confirming the same diagnosis in a larger group of clinically similar cases observed in connection with the above.

Attention is called to the epidemiologic and prophylactic importance of establishing, by close study of such cases, some clinical criteria for their better recognition.

32 (557)

Pure cultures of parasitic amebas on brain-streaked agar.

By ANNA W. WILLIAMS.

[*From the Research Laboratory, Department of Health, City of New York.*]

This work was begun with the attempt to obtain cultures of bacteria-free amebas in crushed rabies brains, the idea being either that the amebas might take up the rabies organisms as food or that they might free the rabies organisms from their host cells and thus bring about, in some way, a culture of the latter, as Clegg reports having done with the *B. leprœ.*

Four cultures of amebas were selected for a first trial, 3, presumably parasitic (type, *Entameba coli*), the fourth, a saprophyte (*Ameba lirnax*). Of these four, only one, a culture from a case of

human amebic dysentery, grew with comparative ease and some abundance on agar plates streaked with rabies brains.

I then tried normal brains as controls and found that this strain of ameba grew with almost equal ease on them; so that the problem was changed for the time from that of a possible aid in determining the nature of the Negri bodies to that of the growth of pure cultures of amebas.

Only four investigators (Kartulis 1893, Casagrandi & Barbagallo 1897, Tsugitani 1898) have claimed to obtain amebas growing free from other living organisms and none of these have been quite clear in regard to their technic nor have they apparently grown their organisms for more than two or three culture generations, which, as controls show, does not rule out the factor of subsistence upon food stored up in the amebas themselves.

The majority of investigators who have tried to obtain pure cultures of amebas all make emphatic statements as to the impossibility of obtaining such cultures by any methods tried.

The present work, the details of which will be given in the full report, may be divided into 3 parts: (1) Obtaining living amebas free from other living organisms, (2) Obtaining sterile brain tissue, (3) Making successive transplants of amebas and brain tissue and proving that every transplant is free from other living organisms. Each step requires many controls.

In obtaining living amebas free from other organisms a method has been used which has been employed successfully by other investigators, differing only in detail. The stock culture of ameba and bacterium is planted in small amount in the center of a nutrient agar plate from which the water of condensation has been dried. On such a plate at 25° to 30° C., in 48 hours, the amebas usually spread beyond the bacteria. If they do not, a drop of normal salt solution will help them do it in another 24 hours, and there is then a wide-spreading zone of motile amebas minus bacteria. Platinum loopfuls of these edge amebas are transplanted to a series of agar plates, and thoroughly controlled for presence of bacteria.

The brain tissue is obtained by removing under strictly aseptic precaution the cerebrums of normal rabbits or guinea pigs, dropping them into 5 per cent. carbolic acid for one to two minutes, then placing them on agar plates and removing Ammon's horns

and corpus striatums which are spead over a series of fresh agar plates, and put in the thermostat for 24 hours. It is peculiarly important to have conditions such as to insure sterility of this tissue medium because of the possibility of the amebas using up any organism that might get into the plates before it could be detected.

This crushed sterile brain is now spread in small amounts over the plates containing the amebas, and placed, some in thermostat, and some at room temperature. If in 24 hours the amebas show slight or no growth as may be the case in the first culture generations, a loop or two of sterile normal salt solution may be added, then in 24 hours at 36° C. or 3–5 days at 20° C., there is generally an abundant growth.

To rule out the presence of extraneous organisms, at each transfer three or four plates are made. To one is added sterile normal salt solution, to another sterile broth, to another sterile blood, one is left simply with the transplanted material, and to the others are added the fresh sterile brain tissue for carrying on the culture. In the second or third culture generations, the cultures transferred on blood or broth or normal salt or plain agar encyst or die out. Spreads, making an added important control, have been made at practically every transfer and usually stained in three different ways. Two other strains of parasitic amebas are being tried and they seem to be growing, though it is too soon to make a positive statement. Liver tissue and spleen tissue are being tried, and the amebas seem to be growing on the liver tissue and not on the crushed spleen.

On the brain-streaked agar plates the one strain of ameba is now growing abundantly in the fourteenth culture generation, with normal brain and in the twentieth culture generation with rabies brain, grown at 36° C. and transplanted every 2–5 days.

33 (558)

A comparison of the growth of sarcoma and carcinoma cultivated in vitro.[1]

By **R. A. LAMBERT** and **FREDERIC M. HANES.**

[*From the Department of Pathology, College of Physicians and Surgeons.*]

We have used in this study a rat sarcoma, a mouse sarcoma and a mouse carcinoma, transplantable tumors of a high degree of virulence. The technique has been practically the same as that employed by Burrows in the cultivation of the tissues of chick embryos and subsequently by Carrel and Burrows in the growth of various mammalian tissues; that is, plasma was obtained by centrifugalizing fresh blood under conditions that prevent coagulation and allowing it to clot in hanging drops which contained small pieces of tumor tissue. These preparations were incubated at 37° C. We have found that rat sarcoma grows in both rat plasma and mouse plasma, and that this is also true of mouse sarcoma. The growth in both instances, however, seems to be more vigorous and of longer duration when homologous plasma is used.

For the character of the growth in vitro, a description of the growth of mouse sarcoma in mouse plasma will suffice. The edges of a piece of sarcoma embedded in plasma are at first fairly uniform in thickness and the piece of tissue is dense and opaque. After twelve hours of incubation at 37° numerous elongated cells project from all sides of the tissue and these wander out into the surrounding plasma by amœboid motion. The throwing out of pseudopods and the associated streaming of the protoplasm can be seen quite beautifully under the microscope. As the cells wander from the original piece of tissue, it becomes less dense and we have observed very frequently the reduction of the original piece of sarcoma tissue to only a fraction of its initial size. Indeed it may become after several days entirely resolved into its component cells, which wander further and further toward the periphery of the plasma. These migrating cells rapidly fill themselves

[1] This investigation has been conducted under the George Crocker Special Research Fund.

with small droplets of fat. This amoeboid wandering of cells is a striking phenomenon in the cultivation of sarcoma in vitro but mitotic division of these cells is frequently seen in stained preparations. "Ring" formation, as described by Harrison, is often observed especially when mouse sarcoma is cultivated in rat plasma. Within the ring where fibrin is absent the cells are seen growing along the cover glass.

The growth of carcinoma in vitro differs quite markedly from that of sarcoma. At the end of eighteen or twenty-four hours there is noted around the piece of incubated tissue a narrow fringe of polygonal cells with large, distinct nuclei. This change is associated with a general flattening out of the specimen. During the next few days this fringe becomes a wide sheet of cells, spread out in a single layer, surrounding the original piece. The edge of this sheet of cells presents an irregular protoplasmic border with moving processes,—a picture almost identical with that described by Harrison for the growth of the epithelium of frog embryos cultivated in frog's lymph. In some preparations groups of cells invade the plasma at certain points, forming what might be termed "alveoli," with the fibrin network as a stroma. In addition to the growth just described, there may be, particularly during the first twenty-four hours, a migration of irregularly shaped cells, similar in type to those seen in sarcoma. Further study will be necessary to determine whether these are carcinoma cells or stroma cells. In stained specimens of growing carcinoma, we have observed mitotic figures at various periods up to the fifth day of incubation. We have not studied specimens of longer duration.

This study seems to demonstrate how closely the character of the growth of tumor cells in the body may be simulated when these tissues are cultivated outside the body.

34 (559)

A respiration calorimeter of the Atwater-Rosa-Benedict type designed for use with dogs and children; with demonstration.

By **H. B. WILLIAMS.**

[*From the Physiological Laboratory, Cornell Medical College, New York City.*]

The apparatus demonstrated is in many respects a miniature of the calorimeters of this type which have been constructed at the Nutrition Laboratory in Boston. The writer wishes to acknowledge his great indebtedness to Dr. Francis G. Benedict, director of that laboratory, for his invaluable assistance in working out the problem of a small calorimeter.

In order to measure with a satisfactory degree of precision the gaseous and energy metabolism of infants and small animals, it has been necessary to introduce some special modifications. As a detailed description of these modifications with a report of the control tests of the apparatus will be communicated within a short time, particular mention need not be made at present.

35 (560)

The chemical and energy transformations in the dog after the ingestion of different quantities of meat.

By **H. B. WILLIAMS, J. A. RICHE** and **GRAHAM LUSK.**

[*From the Physiological Laboratory, Cornell Medical College, New York City.*]

A dog weighing about 13.8 kilograms was given on different days, 700 grams of meat and 1,200 grams of meat at the noon hour. In the morning the metabolism was determined for an hour, while the dog slept quietly in the respiration calorimeter. The minimum or basal metabolism thus determined was found to be about 25 calories per hour. After the ingestion of meat at noon, the animal was again placed in the respiration calorimeter, and the hourly metabolism determined. The results are given in the following table.

HEAT PRODUCTION AFTER MEAT INGESTION.

700 grams meat at noon.

	A. M. 9.45–10.45	P. M. 12.45–1.45	1.45–2.45	2.45–3.45	3.45–4.45	4.45–5.45	5.45–6.45	6.45–7.45
Calories found	25.06	30.78	35.07	33.98	34.09	33.87	33.51	33.49
Calories calculated	26.32	31.33	35.16	33.62	32.41	32.49	31.88	33.45
Grams urinary N.....	0.234	0.591	0.812	0.974	1.52	1.55	1.54	1.25
No. of experiments	(3)	(2)	(1)	(2)	(1)	(1)	(1)	(1)

1,200 grams meat at noon.

Calories found.....	25.06	33.28	33.96	41.52	39.59	38.16	43.43*
Calories calculated	26.32	38.87	40.30	42.53	40.47	40.64	44.15
Grams urinary N.....	0.272	0.865	1.724	1.80	1.88	1.99	1.93
No. of experiments....	(3)	(4)	(2)	(2)	(2)	(1)	(1)

*Dog awake part of the time.

It will be noticed that the metabolism rises from 25 calories in starvation, to 34 calories after the ingestion of 700 grams of meat, and from 25 calories to 40 calories, after the ingestion of 1,200 grams of meat. In general, the figures agree with Rubner's conception, that about 30 per cent. of the total energy of metabolized protein is wasted as free heat, within the organism. One new point stands out prominently in the above table, and that is, that for the first time, the direct and the indirect calorimetry in hourly periods have been found approximately to agree.

An exception to this occurs during the first two hours after the ingestion of 1,200 grams of meat. Here, the calories found are much smaller than the calories calculated by the Zuntz method of indirect calorimetry, that is from the carbonic acid excretion, the oxygen absorption and the nitrogen elimination. This indicates that the method of indirect calorimetry is not an accurate measure for the heat production during the early hours after the ingestion of meat in large quantity.

Two causes may here be active. Either (1) there is oxygen storage within the organism and carbonic acid elimination from the blood, or (2) there are oxidative processes which require oxygen and cause the liberation of carbon dioxide as the result of the production of early cleavage-products of protein metabolism, products which yield carbonic acid and require oxygen, but which do not liberate heat in the same quantities as it is liberated when the protein molecule is oxidized in its entirety.

36 (568)

The metabolism, directly determined, of healthy children during sleep.

By JOHN HOWLAND.

[*From the Department of Physiology, Cornell Medical College, New York.*]

Through the kindness of Dr. Lusk these experiments were made in conjunction with him, Dr. Williams and Mr. Riche.

As yet experiments have been made on only two children, but the results are given on account of the interest attaching to the first direct determination of heat produced by children.

The children were healthy, were gaining weight and were fed every four hours so as to keep their metabolism as constant as possible. They were put on the portable metabolism apparatus devised by Dr. Du Bois that allows great freedom, accurate collection of all the urine and feces and no discomfort. They were kept awake during the forenoon, fed at 1 p.m. and put at once into the calorimeter where they usually slept throughout the whole experiment.

Some of the results are given in the chart. It will be seen that the amount of heat produced varied considerably from hour to hour, and that during the same hours of different days with a constant diet the heat produced is not the same. The average for the hours in which the conditions were entirely satisfactory was 14.18 calories. The metabolism directly determined compared moderately with the metabolism as calculated in about half the cases. In the other half of the cases a low respiratory quotient indicated an erroneous oxygen determination, which invalidated the calculations.

Comparing the results in the two children it is found that the second, McG., regularly gave off more heat than the first (Newman) and that his heat production per square meter of surface per 24 hours was much greater. This is undoubtedly due to the greater surface area of the second child which fails to show by the ordinary formula for calculating the surface area ($12.3 \times \sqrt[3]{\text{weight}^2}$). Tne second child, McG. was 8 months old, weighing 4.320 kilos,

450 grams more than the first, Newman, who weighed 4.770 kilos, and he was 3½ cm. longer, but as the formula contains only one variable and that the weight, it gives a surface area for the second child less than for the first, though it is undoubtedly greater. The formula is apparently very accurate for well-nourished infants but not for the long and poorly nourished.

The heat directly determined and calculated for a square meter of body surface in twenty-four hours was in the two cases 994 and 1,093 calories. These figures correspond closely with the calculated heat in three of Rubner's cases, viz., 1,006, 1,143 and 1,090. The average CO_2 per square meter per hour was: Newman, 15.24; McG., 17.19.

HEAT PRODUCED BY INFANTS.

Newman (3 mos.)

1.45–2.45 P. M.		2.45–3.45 P. M.		3.45–4.45 P. M.	
Calculated.	Found.	Calculated.	Found.	Calculated.	Found.
17.17	17.01	15.96	13.62	15.86	14.91
		15.31	15.72	15.89	13.05
		18.16	13.85		
		13.06	13.42	17.47	13.48
		17.82	15.05	15.31	14.29
McG. (8 mos.)					
15.87	15.19	16.28	14.57	16.66	15.18
				18.02	15.64

37 (562)

Studies on human nephritis.

By **F. S. MEARA** and **A. I. RINGER.**

[*From the Department of Therapeutics of the Cornell University Medical College.*]

PRELIMINARY REPORT.

The object of this investigation was to determine the functional capacity of the kidneys of nephritics with regard to their power of eliminating nitrogenous material, water and salts, and to determine the influence of the "protective therapy" on the kidney efficiency.

We have up to the present the reports of nine cases that have been investigated for periods of two to ten weeks.

The patients after admission to the ward, except when an immediate change in diet was necessary, were placed on a general hospital diet for about three days. The urine and feces were collected and analyzed. After this preliminary test the patients were placed on a diet composed of two quarts of milk, with enough cream and sugar to supply the necessary calories. This diet contained 12 to 13 grms. of nitrogen, 3 grms. of NaCl and about 1,500 c.c. of water. A normal individual should reach nitrogenous equilibrium on about the third day of its administration. Several of our nephritic cases behaved in the following manner:

Case.	Duration of Period.	Average Daily N Intake "A."	Average Daily N Output in Urine "B."	B/A Per Cent.
E.B.	11 days.	12.5 grm.	8.89 grm.	71.1
W.C.	11 days	12.5 grm.	6.91 grm.	55.3

The relationship between the eliminated nitrogen to the ingested nitrogen, expressed in percentages we consider an index of the kidney's nitrogen eliminative power (N.E.P.) when on this standard diet. *A* minus *B* minus the amount of nitrogen found in the feces, represents the amount of nitrogen retained in the system due to the kidney inefficiency. That the nitrogen is not retained because of the building up of protein, but because of kidney inefficiency, is evident from the fact that N/SO_3, ratio in the urine of these cases is very low, and corresponds to the amount of nitrogen ingested.

Case M. with quite marked uræmic symptoms eliminated 4.51, 5.75 and 5.57 grms. of nitrogen per day, with 0.645, 1.113 and 0.97 grms. of inorganic SO_3 for the respective days. The N/inorg. SO_3 ratio during these days was 6.99, 5.17 and 5.72, *i. e.*, the patient eliminated about three times the amount of inorganic SO_3 that is ordinarily eliminated with the same amount of nitrogen.

The N.E.P. of our patients E.B. and W.C. was raised very considerably after several weeks of a "Protective" diet, *i. e.*, a diet containing less nitrogen than the N.E.P. as found in a preliminary test.

The mechanism for the removal by the kidney of one constitu-

ent of the urine may be impaired while the others remain perfectly intact. In case H. with an acute exacerbation of a chronic affection of the kidney due to an attack of tonsilitis, the NaCl eliminating power was absolutely lost. No precipitate could be obtained with $AgNO_3$ after the removal of the albumin, whereas on the same days the patient eliminated water, nitrogen and SO_3 perfectly well.

CASE H.

Date.	Volume of Urine.	Sp. Gr.	Total N.	NaCl	Inorg. SO_3.	$\frac{N}{\text{Inorg. } SO_3}$	Diet.
12/25/10	2405	1010	11.80	0.00	0.8021	14.7	General
12/26/10	2390	1011	11.32	0.00	1.0940	10.3	Salt free.
12/27/10	3120	1007	12.11	0.00	——	——	Salt free.

Similar results were also obtained in case Ba.

38 (563)

A note on the nature of oxyphilic granulation.

By **HERMAN M. ADLER.**

[*From the Danvers State Hospital, Hathorne, Mass.*]

An aqueous solution of eosin will give with ferric chloride a precipitate which has a deep red color. If very dilute solutions are used, say 0.1 of 1 per cent. ferric chloride, and an eosin solution which is just barely pink, no precipitate will be visible on mixing the two, but the pale pink of the eosin changes to a deeper shade of red, and the fluorescence, which is quite noticeable even at this dilution, disappears. If the dilution of the eosin solution used be increased so that these color changes can no longer be distinguished with certainty, it is possible to demonstrate the reaction by the addition of a colloidal suspension of Witte's peptone, or of sodium oleate.

On the addition of such a solution, which should be sufficiently strong to cause a well marked opalescence, the red color of the iron-eosin stain will at once be apparent, especially where sodium oleate has been used, in which case a flocculent precipitate stained a rich red will appear. The examination under the microscope of such a precipitate, reveals an appearance which, in respect to color

is apparently identical with that of oxyphilic granulation. It would appear that we have here not a true stain, in the sense of solution, but rather a case of condensation of the stain on the surface of colloidal particles.

The reaction thus detailed for eosin, is obtained also with Fuchsin S., though the eosin has a more marked color change, due to the loss of fluorescence. The experiment with the dilute solutions is comparable to the conditions under the microscope, where the thin layer of the specimen corresponds to the extreme dilution of the solution, and the visible staining of any tissue indicates a selected accumulation of such stain.

It has been shown that the granules of the eosinophile leucocytes contain iron, and it would seem that the reaction of these granules with eosin and fuchsin was due, in part at least, to this iron content.

The affinity of hæmoglobin for eosin is possibly dependent similarly upon its iron content.

Copper gives a similar reaction. The color change is, however, not quite the same, in that instead of a rich blood-red, the copper-eosin has a distinct purplish or bluish tinge.

Potassium bichromate gives the reaction though very weakly. This is suggestive, perhaps, of the effect of fixation by means of chromates.

In insufficiently stained blood smears, the granules of the eosinophiles, while characteristic in color, are superficially stained.

It is not probable that the stain depends upon a selective affinity, whereby an accumulation of dye is produced within the substance of the granule. It would appear, rather, that this reaction is of the physico-chemical sort, and occurs chiefly at the surface, between the more solid granule and the solution of stain.

39 (564)

Concerning the elimination of dextrose into the gastro-intestinal canal.

By **I. S. KLEINER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Our experiments have reference to statements in literature, some of which, for a better understanding of our results, may be here quoted very briefly. On the basis of analyses of the contents of the gastro-intestinal canal of seven rabbits, Fischer and Moore make the statement that "the small intestine of the rabbit contains no sugar, when an animal is killed shortly after having consumed several hundred grams of carrots and carrot tops or cabbage." J. B. MacCallum reported that in a few experiments with intravenous injection of sodium chloride which, as is well known, produces glycosuria he found some dextrose in the stomach and intestines the quantity of which was slightly increased when, previous to the infusion of the sodium chloride, both kidneys were ligated. MacCallum thought that the presence of glucose in the intestines under these circumstances is due to the hyperglycemia and looked upon it as a sort of "intestinal diabetes"; he believed further that in the absence of the kidneys the intestines assume a supplementary excretory function. Fischer, on the other hand, draws the conclusions from some experiments that hyperglycemia alone does not lead to a secretion of glucose into the intestines. This occurs only, he maintains, when the infusion of sodium chloride is administered to hyperglycemic animals as was the case in MacCallum's experiments on account of the use of morphine.

In our experiments the rabbits had been fed for some time on hay and cabbage. The dextrose determinations were made by Pavy's method and controlled in some instances by Allihn determinations. Various series of experiments were carried out. Only the average figures of each series will be given here. In one series of six normal animals the contents of the stomach and intestines were analyzed some hours after they were fed. The small intestines as well as the stomach contained in each case small amounts of reducing substance. The averages

are: for the intestines 0.05 g. and for the stomach 0.1 g. In a second series six animals received by intravenous infusion 7 grams dextrose per kilo body-weight (in about molecular concentration). The average duration of the infusion was about half an hour; fifteen minutes later the animal was killed, the intestines were washed out and the stomach removed. The averages were: for the intestines 0.16 g. = 1.4 per cent. and for the stomach 0.13 g. = 1.1 per cent. of the injected dextrose. During the period between the beginning of the infusion until the animal was killed for the purpose of washing out the intestines an average of 39 per cent. of the injected dextrose was eliminated in the urine. In a third series of six rabbits only 4 grams of dextrose per kilo were injected (in about 0.6 molecular concentration). The average of this series amounted for the intestines to 0.11 g. = 1.9 per cent., and for the stomach 0.08 g. = 1.1 per cent. of the injected dextrose. Through the urine 23 per cent. of the injected dextrose was eliminated. In the fourth series of rabbits double nephrectomy was performed and on the following day 7 grams of dextrose per kilo were infused. The averages of this series are as follows: for the intestines 0. 24 g. = 2.1 per cent. and for the stomach 0.15 g. = 1.3 per cent. of the injectedd extrose. Finally in a series of only three nephrectomized rabbits the stomach and the intestines were examined on the following day without a preceding infusion of dextrose. Only a mere trace of dextrose could be discovered in these instances. These animals partook of very little food after nephrectomy.

Our experiments have shown that the stomach and especially the small intestines of rabbits contain measurable quantities of dextrose even when the food is poor in easily convertible carbohydrates; that an intravenous injection of dextrose increases perceptibly the gastro-intestinal dextrose even without the aid of a simultaneous infusion of sodium chloride, and that nephrectomy indeed increases the elimination of infused dextrose. However, considering the high elimination of the injected dextrose through the urine, the comparatively insignificant increase of elimination into the gastro-intestinal canal after nephrectomy can hardly be looked upon as a functional assumption of the activities of the kidney, as a vicarious phenomenon.

40 (565)

Experiments with chloroform, administered by intratracheal insufflation, in strychnine poisoning.

By **T. S. GITHENS** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Soon after the introduction of the method of intratracheal insufflation, experiments were begun to study the control of strychnine poisoning by means of this method. So far three series of investigations were carried out. In the first series Shaklee and Meltzer employed, besides insufflation, curare and intravenous infusions of Ringer's solution. In these experiments it was first established that in intravenous injections, 0.4 mgr. of strychnine represents a reliable fatal dose per kilo dog. When using insufflation, curare and Ringer's solution quite a large percentage of dogs were saved which received even more than the fatal dose. Out of 6 dogs which received 0.5 mgr. per kilo, 5 were saved, and out of 22 dogs which received 0.8 mgs. p. kilo 13 were saved.

In a second series of experiments, carried out by the present authors and communicated at the last meeting of the American Pharmacological Society, ether was substituted for curare. Twenty dogs which received 0.8 mgr. strychnine per kilo, that is, twice the fatal dose, were subsequently treated by intratracheal insufflation, ether and intravenous injections of Ringer's solution. All these dogs survived, none died later from after effects and when finally killed the autopsy revealed no abnormal conditions in any of the animals.

In medical practice chloroform is frequently employed in human cases of strychnine poisoning. We have therefore carried out a series of experiments in which chloroform was used instead of ether. We wish to present here the results of these experiments very briefly. Of 21 dogs which received intravenously 0.8 mgr. strychnine per kilo and were treated with chloroform and insufflation 12 survived and 9 died. The contrast to the series in which ether was used is striking. Furthermore, of these dogs only 11 received also infusion with Ringer's solution, 10 did not

receive such infusion. Of the 11 dogs which received Ringer's solution 6 died on the table and 2 died a few days later. Of the 10 animals which did not receive Ringer's solution, 7 survived, two died on the table and one died about 12 hours later. While when using ether the intravenous infusion was a definite favorable factor, it proved to be definitely unfavorable when chloroform was employed. Finally in nearly all the chloroform cases the autopsy revealed pathological conditions, either of the lungs or of the kidneys or of both. The acute deaths were brought about by pulmonary disorders accompanied mostly by pulmonary œdema.

When using the intratracheal insufflation there is no doubt that ether is a safer method than chloroform, at least in the treatment of strychnine poisoning.

41 (566)

A demonstration of osmotic pressure exerted by fat.

By **JACOB ROSENBLOOM** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

In the first of two demonstrations, the authors lowered a cylindrical *rubber* bag, one and one half inches in diameter and eight inches long, into an oiled *muslin* bag of about the same dimensions. The rubber bag was then filled to overflowing with olive oil. The rubber bag expanded, as the oil filled it, to the full length and width of the muslin sheath. The sheath prevented further extension of the rubber bag and imparted rigidity to the osmometer that was ultimately constructed. The full double bag, with its mouth wide open, was then raised so as to enclose about an inch of the lower end of a long glass tube which was firmly supported vertically above the demonstration table. The glass tube was 5 feet long and its bore was 4 mm. in diameter. Ligatures were tightly secured around the neck of the double bag against the immersed lower end of the vertical tube. The bag then hung directly from the end of the tube. The bag and its sheath were in a tightly distended condition and a station-

ary column of oil an inch high in the tube was visible above the protruding edge of the sheath. The tube and bag were then lowered into a salt mouth liter bottle on the table until the bag almost touched the bottom of the bottle. The height of the bottle and the length of the bag were nearly equal. The tube was then marked with a label on the plane of the oil meniscus just above the neck of the bag, and enough ether was poured into the bottle to provide immersion for the bag to the depth of an inch. For a moment no change in the volume of oil was apparent, and the lateral pressure of the ether was obviously without mechanical effect. But in a minute or two diffusion currents were visible along the surface of the bag and oil rose rapidly in the tube.

After the initial effects of the ether had been shown, the bottle was filled with ether containing Sudan III, and a 5-foot vertical extension of the same bore was added to the upright glass tube. In a moment the upward movement of the liquid was accelerated. The demonstration was started at about 9 p.m. At 10 p.m. the osmotic pressure had carried the column of oily fluid to the top of the 10-foot tube, and liquid continued to run rapidly from the upper orifice until the apparatus was dismantled after the adjournment of the meeting, at about 11:30 p.m.

During the progress of the demonstration, Sudan III diffused rapidly from the exterior, through the rubber, to the very top of the rising column of fluid, before any of the liquid passed out of the upper opening. Oil diffused rapidly through the rubber into the ether.

The second demonstration was essentially the same in principle and technic as the first. Instead of a 10-foot upright tube, however, the authors substituted an L tube with an inside diameter of 6 mm. The vertical extension of the tube was 17 in., the horizontal extension was only 3 inches. The latter extension was drawn out to a narrow bore in an inclined plane, to facilitate direct delivery of any liquid that might pass through that end of the tube.

When partial immersion of the bag first occurred there was no visible response, but, in a minute or two, oil began to rise in the tube. The bag was then completely covered with ether. The upward movement proceeded rapidly and in about an hour nearly

200 c.c. of liquid passed through the upper orifice into a graduated cylinder which was supported underneath it to catch the overflow.

The authors are engaged in a study of various relationships that are suggested by the demonstrated phenomena.

43 (568)

A demonstration of the diffusion of pigments from fat through rubber into fat.

By WILLIAM J. GIES.

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

The author has found that many fat-soluble pigments, such as Sudan III and Scarlet R, readily diffuse from solid and liquid fats through rubber into various solid and liquid media, among them both solid fat and oil. Thus, when Sudan III is dissolved in melted lard, the red liquid poured into a rubber bag, the bag supported in melted lard in a bottle, and the apparatus promptly immersed in ice water—the fatty matter will congeal before any sign of pigmentary diffusion occurs, but, in a few hours, a reddish tinge will develop outside of the bag, and on each successive day for several weeks further extension of the pigmented matter may be witnessed, until the whole of the external lard is deeply suffused with red. This process takes place quite rapidly when the lard and apparatus are kept in a thermostat at 40° C.

The demonstrations were intended to exhibit a few instances of such pigmentary diffusions as occur speedily enough at room temperature to yield positive results within an hour. The appended

	Contents of the Rubber Bag.		Nature of the Liquid in which the Bag was Suspended.	Result.
	Oil.	Pigment.		
1	Olive oil.	Scarlet R.	Olive oil	Visible diffusion of the pigment occurred promptly.
2	Cocoanut oil.	Scarlet R.	Cocoanut oil.	Visible diffusion of the pigment occurred promptly.
3	Lard oil.	Sudan III.	Lard oil.	Visible diffusion of the pigment occurred promptly.
4	Paraffin oil.	Sudan III.	Paraffin oil.	Visible diffusion of the pigment occurred promptly.
5	Olive oil.	Sudan III.	Ether.	Visible diffusion of the pigment occurred *almost immediately.*

summary indicates briefly the precise nature and results of the demonstrations (including two control tests—4 and 5), which were made with thin rubber bags in ordinary glass bottles.

The bags were securely supported in the bottles and the mixtures were shaken occasionally during the demonstration. The bags were found, after the adjournment of the meeting, to be without defects.

Numerous related experiments are now in progress.

42 (567)

Comparative dialysis experiments, with demonstrations.[1]

By **F. G. GOODRIDGE** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

When dry bags of rubber, gold beaters' skin, parchment and collodion, each containing olive oil and Sudan III, are separately immersed in olive oil, and the remaining conditions of the environment are uniform, diffusion of the pigment promptly occurs through rubber, but does not take place at all through any of the other three membranes. When the bags are lifted from the oil, washed externally with ether, and then immersed in ether,[2] the pigment quickly passes through the rubber, but diffuses very slowly if at all through the remaining membranes.

Successive immersions of *moist* impermeable membranes (gold beaters' skin and parchment) in alcohol and ether, for different periods of time, fail to render the treated membranes more per-

[1] This and the two preceding communications relate to studies in a projected series on *physico-chemical conditions in the cell*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

[2] In experiments which the senior author has been conducting with Dr. Welker's cooperation, it has been found that collodion bags are disintegrated by *ether containing more than about 1.5 per cent. of alcohol. Pure ether* does not dissolve or in any way disorganize collodion membranes. A collodion bag containing *pure* ether may be immersed for a week or more in *pure* ether without undergoing any appreciable deterioration.

meable to Sudan III under the conditions of the experiments already described.

The authors demonstrated the general facts in this connection pertaining to rubber and gold beaters' skin.

Experiments along these lines, with additional membranes, pigments and liquid media, are in progress, in an effort to obtain further knowledge of the functions of membranes in diffusion.

44 (569)

Occurrence of spontaneous lesions in kidneys and livers of rabbits and guinea pigs.

By W. OPHÜLS.

[*From the Pathological Laboratory of Cooper Medical College, San Francisco.*]

In view of the importance of the occurrence of spontaneous lesions in the kidneys and in the livers of rabbits and of guinea pigs in reference to the experimental work on these organs, Dr. E. C. Dickson and myself have made a careful study of these organs and incidentally of the heart and of the aorta in fifty rabbits and in one hundred guinea pigs. The animals used were partly fresh animals from the market, partly animals raised at the laboratory.

Many of the rabbits had been used in the physiological laboratory and had been killed immediately after the experiments; some died of coccidiosis; a few had been injected with material supposedly containing pneumococci without result; others had never been used. Several old rabbits that had been in the laboratory for a year or longer were especially selected on account of the greater likelihood of the existence of renal or hepatic lesions in them. Twenty-eight rabbits, among them some of the old ones, had entirely normal kidneys, nine showed slight parenchymatous lesions, three a few small areas of cellular infiltration. In ten we found scattered small areas in which were marked interstitial lesions with the formation of small depressions on the surface. Four of these proved to be radially arranged chronic septic foci which extended from the vicinity of the papilla to the outer surface.

In the other six cases the same arrangement of the new formed connective tissue in narrow radial bands which start deep in the pyramids was noticeable although no evidence of a septic infection could be discovered. The interstitial lesions were accompanied by marked epithelial lesions in two cases only, producing to a certain extent the picture of a chronic parenchymatous nephritis, although the lesions were never very extensive. Lesions of the blood-vessels or primary lesions of the glomeruli were not found in any case. The lesions described by the writer in his paper on experimental chronic nephritis[1] in rabbits 52 and 48 are probably spontaneous ones. Only six normal livers were encountered; the rest showed various stages of coccidial infection with more or less cirrhosis and sometimes cirrhotic processes in cases in which coccidia could not be demonstrated. We are very strongly of the opinion that our findings throw very serious doubt on the numerous reports of the experimental production of cirrhosis in the liver of rabbits as this animal would seem to be entirely unsuitable for such experiments. No gross lesions of the heart or of the aorta were discovered in any of the animals.

Of the one hundred guinea pigs the large majority had been injected with sediment from urine or other material suspected of containing tubercle bacilli; a few only actually developed tuberculosis. There were several especially selected old animals. Sixty-three of the one hundred had absolutely normal kidneys, two showed slight parenchymatous lesions and in thirty-five there were scattered small areas of cellular infiltration in the cortex which were sometimes rather numerous, at other times they were found with difficulty only on careful examination of several sections. They consist largely of lymphocytic cells and few larger cells with vesicular nuclei. They do not seem to have much tendency to develop into fibrous tissue although occasionally such a change was observed but always to a limited degree only. A reason for the existence of these foci could not be discovered. The lesions described in the writer's article on "Experimental Chronic Nephritis"[2] in guinea pigs as representing the early stages of renal lesions in chronic lead poisoning are probably of

[1] *Journal of Medical Research*, 1908, XVIII., 497.

[2] *Journal American Medical Association*, 1907, XLVIII., 483.

this character. In few cases fibrous thickening of the capsules of glomeruli and cystic dilatation of the same were observed as spontaneous lesions similar to certain types of glomerular cysts described by E. C. Dickson in his paper on the "Experimental Production of Chronic Nephritis in Animals by the Use of Uranium Nitrate."[1] These were, however, rare exceptions, whereas in uranium poisoning such glomerular lesions seem to be nearly constant. Large scars or lesions of the blood vessels were not found in any case.

Of the livers two showed extensive necroses, a disease probably familiar to all who handle many guinea pigs. In one case there was a slight cellular infiltration of the periportal connective tissue and in two sufficient new formation of connective tissue to speak of it as an incipient cirrhosis. A condition resembling cirrhosis therefore seems to occur spontaneously in guinea pigs also, but, so far as our observations go, it is quite rare. Heart and aorta were carefully inspected in all cases, but nothing abnormal was noted in any instance.

45 (570)

Spontaneous nephritis in wild rats.

By **W. OPHÜLS.**

Among the very many rats examined in San Francisco for plague only very few were found that showed evidences of dropsy and of renal disease. One such rat was carefully examined at our laboratory through the courtesy of the U. S. Public Health and Marine Hospital Service. This rat showed a very marked general oedema. The kidneys are small, distinctly granular. The measurements are 26×14×10 mm. The heart is moderately but distinctly enlarged, measuring in the formalin hardened specimen from base to apex—23 mm., the largest transverse diameter is 19 mm. and the largest antero-posterior diameter 14 mm. There are no gross lesions of the aorta. In sections of the kidneys the blood-vessels are found in a normal condition. The glomeruli are also quite normal except a few which show slight fibrous thickening

[1] *Archives of Internal Medicine*, 1909, III., 375.

of capsule and slight cystic dilatation. The epithelium in practically all tubules is very markedly and extensively degenerated, showing granular and fatty degeneration. There are many casts. Large bunches of peculiar unidentified, uncolored needle-shaped crystals are also found in the tubules. Large areas in the sections show collapse of tubules with much cellular infiltration and new formation of cellular fibrous tissue between them. The appearances are those of a chronic parenchymatous nephritis. It is possible that these lesions are the result of one or the other poison, such as arsenic or phosphorus, which were used in destroying rats in this city. In this connection it is noteworthy however how rarely such lesions were observed in spite of the very extensive use of such poisons.

46 (571)

The stimulation of adrenal secretion by emotional excitement.

By **W. B. CANNON** and **D. DE LA PAZ.**

[*From the Laboratory of Physiology in the Harvard Medical School.*]

Dreyer's demonstration that splanchnic stimulation increased the content of adrenal secretion in blood from the adrenal veins has been confirmed by several observers. Adrenal secretion therefore is under control of the sympathetic system.

Major emotional disturbances indicate the dominance of sympathetic impulses. In the cat, for example, fright causes dilation of the pupils, inhibition of the stomach and intestines, rapid heart, and erection of the hairs of the back and tail. Do not the adrenal glands share in this widespread subjugation of the viscera to sympathetic control?

To try this suggestion the inhibition of contraction in strips of longitudinal intestinal muscle, sensitive to suprarenin 1:20,000,000, was used as a biological test. Blood was obtained from the cat when quiet, and again after the animal was excited by the presence of a barking dog, by introducing, through the femoral vein, into the inferior vena cava to the region of the liver, a small vaselined catheter. The blood thus obtained was defibrinated and applied to the intestinal strip at body temperature.

After an initial shortening the strip contracted rhythmically in blood from a quiet animal. In no instance did such blood produce inhibition. On the other hand, blood taken from animals after the emotional disturbance, showed more or less promptly the typical relaxing effect. As the emotional period was prolonged, the effect became prompter and more profound.

The view that inhibition of the contracting intestinal strip is due to an increased content of adrenal secretion is justified for the following reasons. (1) The effect was obtained in blood from the vena cava near the liver when that from the femoral vein taken simultaneously produced no inhibition. (2) Removal of the adrenal glands after tying the adrenal vessels resulted in a failure of excitement to produce the effect. (3) Adding varying amounts of adrenalin to inactive blood evoked all the degrees of relaxation that have been observed in excited blood. (4) Excited blood which produced prompt inhibition lost that power on standing or on being agitated by bubbling oxygen. These conditions together with the evidence that sympathetic impulses increase the secretion of the adrenal glands, and that during such emotional excitement as was here employed signs of sympathetic discharge were observable in the animal from the eye to the tip of the tail, prove that the effect was due to adrenal secretion.

Injected adrenalin is capable of inducing an atheromatous condition of the arterial wall in rabbits, especially in elderly individuals, and is also capable of evoking hyperglycemia with glycosuria. As Ascher has shown, by prolonged stimulation of the splanchnic nerves, prolonged secretion of the adrenal glands with maintained high blood pressure can be produced. In the light of the results here reported the temptation is strong to suggest that some phases of these pathological states are associated with the strenuous and exciting character of modern life acting through the adrenal glands. This suggestion, however, must be put to experimental test.

47 (572)

The production of glycosuria as a result of the intravenous injection of Witte's peptone.

By YANDELL HENDERSON and FRANK P. UNDERHILL.

[*From the Physiological Laboratory, Yale Medical School and the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

Renewed investigation concerning the phenomena provoked by intravenous administration of Witte's peptone has demonstrated the production of a marked glycosuria, following such injections. The appearance of sugar in the urine is accompanied by hyperglycæmia. The experiments were carried out for the most part upon dogs. When Witte's peptone is injected into the rabbit glycosuria is not in evidence, an observation which is in entire accord with the failure of this substance to induce certain other phenomena in this animal which are brought about in the dog.

The tentative hypothesis is advanced that the presence of sugar in the urine is induced as a result of the respiratory disturbances set up by the "peptone" injection.

The phenomena connected with the injection of "peptone" mixtures are being subjected to further investigation.

48 (573)

Tubercle bacilli in the feces of cattle.

By W. J. MACNEAL and C. F. BRISCOE.

[*From the Agricultural Experiment Station, University of Illinois, Urbana, Ill.*]

Between June, 1908, and August, 1910, a number of tests upon the feces of cattle were made at the Illinois Agricultural Experiment Station to determine the presence or absence of tubercle bacilli. These tests were of two kinds, (1) by direct microscopic examination and (2) by inoculation of animals.

The microscopic method included the examination of stained slide preparations of (*a*) mucus on the exterior of the fecal mass,

(*b*) the mixed feces, and (*c*) watery mucus scraped from the rectum, each stained slide being searched fifteen minutes with the aid of a mechanical stage. If organisms indistinguishable in appearance from bovine tubercle bacilli were found upon any of these slides the examination was recorded as positive. One hundred and nineteen samples from 53 cows which had reacted to tuberculin were examined in this way, with a positive result 53 times in samples from 34 of the cows. Thirty-five other samples from these same cows were negative. Nineteen cows gave negative results at all examinations, 31 in number. Thus, of the 53 cows tested, 34, or 64.2 per cent., gave a positive result at one or more tests, and 19, or 35.8 per cent., gave only negative results. Forty samples from 18 non-reacting cows were tested in the same way, with 23 positive and 17 negative results. Fifteen of the 18 cows, or 83 per cent., gave a positive result at one or more tests, while only 3, or 17 per cent., were negative at all tests. From this it appears that no significance in respect to the presence of tubercle bacilli can be attached to the finding of a few acid-fast bacteria in the feces of cattle.

In order to test the delicacy of guinea pig inoculation as a test for tubercle bacilli in the feces of cattle, a number of experiments were performed in which accurately measured minute quantities of a pure culture of bovine tubercle bacilli were added to definite amounts of cow feces, previously shown to be free from tubercle bacilli. In this way it has been ascertained that as small a quantity as 1×10^{-11} gram of tubercle bacilli, suspended in one cubic centimeter of emulsion of feces, produces generalized tuberculosis upon subcutaneous injections into guinea pigs. The actual number of bacterial cells in this amount of culture would probably be between 5 and 500.

One hundred and four samples of feces from 62 reacting cattle have been tested by inoculation of guinea pigs. Virulent tubercle bacilli were thus detected in feces from three of these animals, or practically 5 per cent. No relation was found between the presence of visible acid-fast bacteria in the microscopic test and the presence of virulent tubercle bacilli as shown by guinea pig inoculation.

Whether the percentage of reacting cattle passing tubercle

bacilli in the feces is relatively large or small would appear to depend upon the stage of the disease in the individual animals. It is important to point out, that even in well-nourished and apparently healthy herds of reacting animals (such as those used in this work) there are likely to be, at all times, a few animals actually passing virulent tubercle bacilli in the feces.

A detailed account of this work is published in Bulletin No. 149 of the Illinois Agricultural Experimental Station.

49 (574)

An alteration of the sex-ratio induced by hybridization.

By **T. H. MORGAN**

[*From the Department of Zoölogy, Columbia University.*]

Males and females of the fly *Drosophila amphelophila* occur in nature in about equal numbers. A long series of experiments failed to induce any change in the sex-ratio by adding different sugars, salts, acids or alkalies to the food on which the larvæ live. A remarkable alteration of the sex-ratio took place, however, in the second generation of certain crosses between two races that arose as mutations. When a male of a race with rudimentary ("short truncated") wings was crossed to a female of a race with short proportionate wings all of the females had normal wings and all of the males short proportionate wings, like the mother. When these (F_1) were inbred, there were produced in the second generation three classes of individuals according to the character of the wings; namely, long (normal), short proportionate, and short rudimentary distributed according to sex as shown below:

Long ♀	Long ♂	Propt ♀	Propt ♂	Rudim ♀	Rudim ♂
989	137	336	389	8	12

The normal males are to the females as one to seven, while in the other two classes the sex ratio is approximately normal. *The point of prime importance is that it is the normal males that are affected.* Even if we assumed that the deficiency might be due to the absence (in part) of males with rudimentary wings, and double the number of males with normal wings, the number of normal males would still fall far below that of the females.

The reciprocal cross, namely, short proportionate males and rudimentary winged females gave, in the first generation, long-winged males and females. These inbred produced in the second generation:

Long ♀	Long ♂	Propt ♀	Propt ♂	Rudim ♀	Rudim ♂
265	80	36	90	0	14

Again, the sex disproportion is observed for the long-winged flies, while the reverse order holds now for the proportionate wings.

The F_2 flies from both of the above sets have been bred again in all possible combinations within the same set, and the disproportion of males to females was found to be transmitted to the next generation, although not in the same ratios. Some of the proportionate winged males (F_2) were also bred to wild flies, and gave 301 females and 111 males.

The most probable explanation of these results would seem to be either in the partial degeneration of the male producing spermatozoa as a result of crossing with the race with rudimentary wings or in some quantitative change in the sex determining factor. The effects are transmitted also to a third generation. Whether they are permanent or not remains to be tested.

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Members elected at the forty-second meeting:

W. F. Longcope, F. M. McCrudden, H. O. Mosenthal, Jacob Rosenbloom.

Dates of the next two regular meetings:

April 19, 1911, at Philadelphia. May 17, 1911.

PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

FORTY-THIRD MEETING

THE LABORATORY FOR HYGIENE
UNIVERSITY OF PENNSYLVANIA

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CONTENTS

The proceedings of the Society for Experimental Biology and Medicine are published as soon as possible after each meeting. Regular meetings of the Society are held in New York on the third Wednesdays of October, December, February, April and May. A volume of the proceedings consists of the numbers issued during an academic year.

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SCIENTIFIC PROCEEDINGS.

Abstracts of the Communications.

Forty third meeting.

The Laboratory for Hygiene, University of Pennsylvania. April 19, 1911. President Morgan in the chair.

50 (575)

On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog (R. pipiens).

By **Henry H. Donaldson.**

[*From the Wistar Institute of Anatomy and Biology.*]

The relative weight of the central nervous system of the frog, *Rana pipiens*, changes during the active season, and such a change is probably characteristic for other species of frogs with like habits.

The relative weight of the central nervous system is low at the time of emergence, high in the midsummer (July) and low again at the time of hibernation. During hibernation it remains nearly constant. In the formula[1] used to express the weight of the central nervous system, the absolute value of C is characteristic for the station from which the frogs come.

The range from minimum to maximum in the value of C is about 13 per cent., rising 7 per cent. from the end of March to the end of April, 4 per cent. more from the end of April to the end of May, and 2 per cent. more from the end of May to the first of July, remaining stationary in July and then falling month by month at a similar rate to the end of October.

This variation in the relative weight according to season is due to lack of coincidence between the growth of the central nervous system and the growth of the entire body.

[1] This formula is as follows: Weight of C. N. S. = (log. $W.\sqrt[4]{L.}$) C indicating that the weight of the central nervous system is equal to the log. $W.$, body weight in grams, by $\sqrt[4]{}$ of the total length L, in mm., this product to be multiplied by C, a constant.

In frogs from one to four years old, the body weight more than doubles during each active season, although the precise form of the curve representing this body growth is not known.

The growth of the central nervous system is precocious in relation to that of the body, but in the absence of direct observations on the growth of the body, the form of the curve can only be indirectly determined.

During the active season, the percentage of water in the entire frog falls slightly from spring to summer and rises again from summer to autumn. These changes seem to be due to the combined effects of advancing age and varying food supply.

51 (576)

An interpretation of growth curves from a dynamical standpoint.

By **S. HATAI.**

[*From the Wistar Institute of Anatomy and Biology.*]

The growth phenomena may be considered as a gradual transformation of growth energy to the work done in forming the mass which composes the body. The present writer wishes to determine "what law, if there is any, governs in any individual the rate of transforming the growth energy into the work done."

In order to solve the above problem, the assumption was made that under normal conditions the growth energy is transformed into the work with least loss of energy. It was shown that in order that this assumption should be true we must have $\delta A = 0$ in the following integral

$$A \text{ (action)} = \int mvds.$$

Applying this principle it was proved that the formula for the growth of brain in weight (and any other data which satisfy the same conditions) must be a function which renders the following integral minimum

$$u = k\int\left(\frac{1}{x}+\frac{1}{a}\right)\sqrt{1+\left(\frac{dy}{dx}\right)^2}\,dx.$$

The integral is minimized when the function y has the following relations:

$$y = \frac{ca}{k\left(1 - \frac{c^2a^2}{k^2}\right)^{\frac{1}{2}}} \sqrt{\left(x + \frac{a}{1 - \frac{c^2a^2}{k^2}}\right)^2 - \frac{c^2a^4}{k^2\left(1 - \frac{c^2a^2}{k^2}\right)^2}} - \frac{ca^2}{k\left(1 - \frac{c^2a^2}{k^2}\right)^{\frac{3}{2}}}$$

$$\times \log \left\{ \left(x + \frac{a}{1 - \frac{c^2a^2}{k^2}}\right) + \sqrt{\left(x + \frac{a}{1 - \frac{c^2a^2}{k^2}}\right)^2 - \frac{c^2a^4}{k^2\left(1 - \frac{c^2a^2}{k^2}\right)^2}} \right\} + c_2.$$

If our assumptions are correct, the above formula ought to represent adequately our growth curves. It was shown that the above formula can be transformed into the following forms as particular cases:

$$y = a + b \log (x + c),$$
$$\text{and } y = a + bx + c \log x.$$

These are formulas which are already extensively used for graduating observed growth curves. Thus there is no further need to test the adequacy of the formula to represent the growth curves, since numerous applications have been made with satisfaction and already published by various investigators. I therefore put forward the following provisional definition of growth considered as a process: "An organism tends during growth to form greatest amount of mass with least loss of growth capacity."

Further the present investigation furnishes a biological meaning to the logarithmic formulas which have been extensively used without appreciating the full significance of their properties.

The cases of abnormal growth were also discussed but will be treated more fully in a future publication.

52 (577)

Experiments to modify the sex ratio in the toad.

By HELEN DEAN KING.

[*From the Wistar Institute of Anatomy and Biology.*]

Several series of experiments were made in the spring of 1910 in order to ascertain whether the sex ratio in the toad can be altered by subjecting the eggs to different environmental conditions at or before the fertilization period.

Lots of eggs fertilized in various solutions of alcohol (.13 per cent. to 2 per cent.), as well as those fertilized with sperm from

the right or from the left testicle of three different males, all gave practically normal sex ratios as the proportion of females ranged from 46.92 per cent. to 55.64 per cent.

Three batches of eggs from two different females were fertilized out of water and kept in a moist chamber for several hours. Each batch of eggs gave an unusual excess of females, the proportion of females varying from 60.86 to 70.83 per cent.

Mature eggs from another female were subjected to the action of a 2.5 per cent. solution of salt or of cane sugar for ten minutes and then fertilized in tap water. In each case 70 per cent. of females was obtained. In this series of experiments, as well as in the preceding one, it is probable that the eggs lost water during the fertilization period or at least were prevented from absorbing water during this time.

Seven lots of eggs from four different females were fertilized in solutions of hydrochloric or of acetic acid, the strength of the solutions varying from .01 per cent. to .0025 per cent. In every instance the percentage of females obtained was from 10 to 20 per cent. lower than that which is probably normal for the species. Lots of eggs from the same females fertilized in alkaline solutions (NH_4OH or NaOH) of the same strength gave proportions of the sexes within the range of probable normal variations in the sex ratios of different lots of individuals. It is probable that the acid solutions caused the eggs to absorb an extra amount of water during the fertilization period. The alkaline solutions were apparently too weak to have any influence on the eggs.

No definite conclusions can be drawn from these experiments, since in every case the mortality was very great. The results strongly suggest, however, that in the toad, *Bufo lentiginosus*, sex is determined at or near the time of fertilization and that it can be influenced by external factors. They also seem to indicate that the relative amount of water in the egg at the time of fertilization has some influence in determining sex: an increase in the water content tending to produce a male; a lowering of the water content favoring the development of a female.

53 (578)

Evidence that the primary change in stimulation is an increase in the permeability of the limiting membranes of the irritable elements.

By **RALPH S. LILLIE.**

[*From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory, Zoölogical Department, University of Pennsylvania.*]

Various facts and theoretical considerations indicate clearly that the process of stimulation in muscle or nerve has its seat at the semi-permeable boundary layers or plasma membranes of the irritable elements, and consists in a sudden and reversible increase in the permeability of these membranes. After a brief review of this general evidence the following experiments were described.

I. *Experiments with the Larvæ of Arenicola cristata.*—These are the free-swimming ciliated larvæ of a marine annelid; they are small worm-like organisms about 0.3 mm. in length, readily obtained in large quantity by rearing. The larvæ have a well-developed muscular system; the special peculiarity which fits them for the purpose of the following experiments is the presence throughout the whole body of a water-soluble yellow pigment; this substance is contained *within the cells*, and does not visibly leave the latter except under conditions of markedly increased permeability—as on death or after treatment with cytolytic substances (*e. g.*, saponin); it then diffuses into the medium and, if sufficient larvæ are present, colors the latter a bright straw yellow. Its exit thus serves as a convenient index of increased permeability. It was found that during strong chemical stimulation a rapid loss of pigment always occurs: the rate and degree of this loss run closely parallel with the intensity of the stimulating action, as indicated by the extent and duration of the muscular shortening. Pure isotonic ($m/2$) solutions of neutral sodium salts ($NaCOOCH_3$, NaCl, NaBr, $NaNO_3$, $NaClO_3$, NaI, NaCNS) all cause strong and persistent muscular contraction accompanied by rapid loss of pigment; the addition of a little calcium chloride to the solution (1 c.c. $m/2$ $CaCl_2$ to 25 c.c. $m/2$ sodium salt) prevents both the

stimulating action and the loss of pigment. Potassium salts show a similar stimulating and permeability-increasing action, neither of which, however, is checked by the addition of calcium. Isotonic LiCl solution shows moderate stimulation with moderate loss of pigment; both are checked—as in the case of NaCl—by calcium. On the other hand while pure *m*/2 CsCl produces well-marked stimulation and loss of pigment, the addition of calcium does not check, but on the contrary markedly accentuates both effects. Mixtures of potassium and magnesium chlorides show varying action according to the relative proportions of the salts; in pure isotonic $MgCl_2$ solution there is neither stimulation nor loss of pigment, but complete reversible muscular anæsthesia; the same is true of mixtures containing a decided excess of $MgCl_2$ (*e. g.*, 1 volume *m*/2 KCl+4 volumes *m*/2 $MgCl_2$); when the proportion of KCl is increased to one half or more, stimulation and with it loss of pigment appear; in mixtures of equal parts both effects are slight; in mixtures of 2 vols. *m*/2 KCl to 1 vol. *m*/2 $MgCl_2$ both are somewhat increased; in a mixture of 4 vols. *m*/2 KCl to 1 vol. *m*/2 $MgCl_2$ there is moderate stimulation with moderate loss of pigment, though both effects are decidedly less marked than in pure *m*/2 KCl. Saturated solutions of chloroform or ether in sea-water produce strong contraction with rapid loss of pigment; weak solutions anæsthetize without stimulating or visibly increasing permeability.

II. *Experiments with Frog's Muscle.*—It was pointed out that many cytolytic substances produce slow and usually irreversible contraction in vertebrate skeletal muscle; this is the case with soaps, bile-salts, various hæmolytic alkaloids or glucosides (*e. g.*, saponin, digitalin, solanin, agaricin), strong solutions of lipoid solvents (chloroform, ether, benzol, toluol, etc.), certain foreign blood sera and certain bacterial toxins (*e. g.*, tetanus). The contraction is typically slow and steady, unaccompanied by twitching, and passes over into permanent rigor. It was found that after treatment of the muscle for some minutes with pure isotonic solutions of various neutral sodium salts the response to many of the above substances is so altered that rapid and vigorous contractions with twitching may result. This sensitizing action increases in the order: NaCl, NaBr, $NaNO_3$, $NaClO_3$, NaCNS and NaI, being usually slight

with the first two salts, and well marked with the others, particularly with iodide and sulphocyanate. Frog's gastrocnemii immersed for five minutes in *m*/8 NaI or *m*/8 NaCNS show rapid contraction and twitching when immersed in isotonic solutions of the following substances in Ringer's solution or physiological salt-solution: saponin, digitalin and solanin (marked action); agaricin and aconitine (relatively slight action); chloroform (marked action); Na-oleate (marked action); bile-salts (marked action); horse and dog serum (marked action with vigorous twitching); tetanus toxine (vigorous twitching); rattlesnake venom (moderate action). The intensity of the stimulating action shows a general parallelism with that of the hæmolytic action.

Muscles may be similarly sensitized to osmotic stimuli (distilled water and hypertonic sodium chloride solution). This fact, as well as the fact that colloidal substances (serum, etc.) may show marked stimulating action, furnishes additional proof that stimulation depends essentially on an alteration of the plasma membrane.

54 (579)

Nature of the muscular contraction.

By **E. B. MEIGS.**

[*From the Wistar Institute of Anatomy and Biology.*]

The comparison of histological preparations of uncontracted and contracted smooth muscle indicates that during the contraction of this tissue fluid passes from the fibers to the interstitial spaces. It seems possible, therefore, that the contraction of smooth muscle may be brought about by an interchange of fluid between its cells and their surroundings in the same way that the movements of *Mimosa* are caused by changes in the turgor of its cells. This hypothesis may be tested by investigating the effect of swelling reagents and their opposites on the length of smooth muscle. The hypothesis would be supported if it could be shown that smooth muscle lengthened when immersed in solutions which cause it to gain in weight and shortened in the opposite class of solutions.

The changes of weight and the changes of length of frog's smooth muscle have been followed in Ringer's solution, in various

modifications of Ringer's solution, in 0.7 per cent. NaCl solution, in isotonic saccharose, glucose, glycerine and alanin solutions. In all the cases investigated it has been found that increase in weight goes hand in hand with increase in length and that decrease in weight is accompanied by decrease in length. The changes in weight and the changes in length are roughly proportional to each other in rate and amount.

A suggestion of the nature of the chemical change which brings about contraction is found in the following facts. Smooth muscle usually gains in weight and lengthens in Ringer's solution which has the following formula: NaCl, 0.65 gram; KCl, 0.02 gram; $CaCl_2$, 0.025 gram; $NaHCO_3$, 0.02 gram; H_2O, 100 c.c. If for the $NaHCO_3$, 0.01 gram of lactic acid be substituted, the gain in weight and lengthening do not occur; there may even be a small loss in weight and shortening. Larger amounts of lactic acid may cause gain in weight and lengthening. If the muscle be stimulated with a strong interrupted Faradic current after it has been for some time in the weakly acid Ringer, it responds to the stimulation by lengthening slightly instead of by shortening. These facts may be explained by supposing that smooth muscle responds to stimulation like striated muscle by the production of a small amount of lactic acid. Under ordinary circumstances this production of lactic acid causes the fibers to lose fluid and shorten; but if the fibers have been artificially acidified before the stimulation takes place, the production of more acid on stimulation causes the fibers to take up fluid and lengthen.

55 (580)

Preliminary note on the action of some internal secretions upon erectile tissue.

By **ISAAC OTT** and **JOHN C. SCOTT.**

[*From the Medico-Chirurgical College of Philadelphia.*]

To study the action upon erectile tissue we employed the penile organ of the dog. The length of it was measured by a pair of compasses, from the bulb to the tip. The width of the bulb of the organ was measured in the same way. Then the filtered infusion made with distilled water was injected into a vein and the

dimension of the organ measured as before. It was found that the prostate increased the length 25 mm., and the width of the bulb 25 mm. The orchitic extracts gave an increase in length of 15 mm., and an increase in width of bulb of 23 mm. The ovarian extract gave an increase in length of 21 and an increase in width of bulb of 23 mm. The parathyroid extract gave an increase in-length of 15 and an increase in width of bulb of 15 mm. The thymus extract gave an increase in length of 15 mm. and an in crease in width of bulb of 12 mm. Pituitary extract as a whole, gave an increase in length of 11 mm. and an increase in width of bulb of 6 mm. Infundibulin gave an increase in length of 5 mm. and an increase in width of bulb of 6 mm. Pineal gland gave no increase in length but increase in width of bulb of 7 mm. The corpus luteum increased the length 15 mm. and the width of the bulb 28 mm.

56 (581)

The depressor action of dog's pancreas and pancreatic juice.

By **A. B. EISENBREY** and **R. M. PEARCE.**

[*From the Laboratory of Research Medicine, University of Pennsylvania.*]

The marked lowering of blood pressure produced by the pancreatic juice of the dog when injected into the circulation of the dog, suggested that the sudden onset of prostration and collapse, symptoms indicative of vasomotor insufficiency seen clinically in acute pancreatic disease, might be due to the direct peritoneal absorption of the pancreatic juice set free in the destruction of the gland.

We have accordingly collected the pancreatic juice from anesthetized dogs by placing a canula in the duct, and the secretion so obtained was diluted with equal volumes of 0.85 per cent. salt solution and injected into the femoral or external jugular veins of the same or other dogs. Varying with the anesthetic used and directly with the blood pressure level at the time of injection, we have obtained falls of from 20 to 60 mm. Hg by injecting the equivalent of 1 c.c. of pancreatic juice. The action does not appear to depend on any primary cardiac effect. The fall is prompt and recovery to the original level occurs within 2 minutes. Pan-

creatic juice from which the coagulable proteins were removed by heat caused an equally marked fall in the blood pressure.

Extracts of the fresh pancreas were also prepared with the Buchner press or by simple grinding in a mortar with sand or glass and shaking with 0.85 per cent. salt solution. As a general rule 3 c.c. of the resulting solutions representing 1 gm. of the organ were used for single injections. The results were similar whether the solution was used at once or was kept on ice for 18 to 24 hours. Before use the solutions were cleared as far as possible by centrifugalization and filtering. Such extracts produced a fall in blood pressure averaging 30 to 50 mm. Hg. Similar results were obtained by using extracts from which the coagulable proteid was removed by heat and acetic acid, and also with the alcoholic precipitate of such a solution taken up in the original volume of 0.85 per cent. salt solution.

The alcoholic filtrates of the preceding extracts evaporated to dryness at 37.5° C. and made up to the original volume are inert. Washed or unwashed organs give extracts of similar depressor activity. The clear solutions obtained after heating and acidification were used to determine the effects of the injection of small amounts of extract over long periods of time. Death resulted generally from the injection of the equivalent of 3.5 to 6 grams of pancreas over a period of about one half hour, but in one instance an animal received the equivalent of 31 grams of pancreas, during two and one half hours, before death occurred.

It was found impossible by continuous injection of small quantities to keep the blood pressure at the low level produced by a single injection of the equivalent of 1 gram of pancreas. The blood pressure remains but slightly below its original level, but if a large dose is then given there is a marked fall, with recovery to the level existing at the time of injection. Attempts to determine the effects of continuous absorption of pancreatic juice from the peritoneal cavity by causing leakage from the severed ducts were also unsuccessful.

That there might be some ground for drawing analogies between experimental findings and clinical conditions, extracts were prepared from human pancreas and it was found that their action on the dog was in every way similar to that obtained with

the pancreas of the dog. We have, however, from our experiments no evidence to show that the prostration and collapse of acute pancreatic disease are caused by the absorption and effect on the blood pressure of the unaltered pancreatic juice or the products of the earlier stages of autolysis. The part played by autolysis in the production of further depressor and toxic substances and especially the part played by the activation of the pancreatic secretion by the enterokinase, which body presumably finds entrance into the organ in the development of the human lesion, are phases of the subject which we now are investigating.

57 (582)

The significance of the structure of the medullary loop of the renal tubule of mammalia.

By G. CARL HUBER.

[*Laboratory of Histology and Embryology, University of Michigan.*]

A method of maceration has been devised recently[1] by means of which it is possible to isolate the entire renal tubule of adult mammals. Certain of the renal tubules thus isolated have been stained and permanently mounted in glycerine. This enables a study of their form in a manner hitherto not possible and admits of an accurate determination of their epithelial lining. Each mammalian renal tubule possesses four types of epithelium: (1) The pavement epithelium surrounding the glomerulus and lining the glomerular capsule; (2) the specific renal epithelium of the proximal convoluted portion and its medullary segment; (3) the pavement epithelium of the medullary loop; (4) the cubic or short columnar epithelium of the ascending or distal arm of the medullary loop and the distal convoluted portion. The following table shows the distribution of the last three types of epithelium in renal tubules of the rabbit, the tubules selected representing A, a tubule with renal corpuscle situated at the periphery of the cortex; B, a tubule with renal corpuscle situated in the deeper portion of the outer half of the cortex; C, a tubule the renal corpuscle of which is situated in the deepest part of the cortex.

[1] G. Carl Huber, "A method for isolating the renal tubules of mammalia," *Anat. Record.*, v., April, 1911.

	A, mm.	B, mm.	C, mm.
Proximal convoluted portion with medullary segment..... (Specific renal epithelium of cortex.)	11.3	9.4	10.2
Thin segment of medullary loop..... (Pavement epithelium.)	1.4	6.7	15.0
Thick segment of medullary loop and distal convoluted portion..... (Cubic or short columnar epithelium.)	7.8	6.9	3.6
Total length of tubules, exclusive of collecting duct.	20.5	23.0	28.8

The tubules selected represent type tubules and were selected with a view of drawing attention to the fact that the character of the tubule, and perhaps also its functions, changes with the position of the renal corpuscle in the cortex. Tubules with renal corpuscles situated near the periphery of the cortex possess short medullary loops with short segments lined by pavement epithelium. The deeper in the cortex the renal corpuscle is situated the longer becomes the medullary loop and the longer the segment of the loop lined by pavement epithelium. These statements pertain not only to the renal tubules of rabbits but also to those of other mammals including man. It may be observed from a study of the table that that portion of the renal tubule lined by the specific renal epithelium of the cortex, namely the proximal convoluted portion and its medullary segment, does not vary materially in length in tubules of different types. If one may assume a specific excretion for the special renal epithelium of the proximal convoluted portions and their medullary segments, it is evident that this specific excretion would be essentially of the same extent for all the renal tubules, irrespective of type. If, on the other hand, one may assume an absorption of water and perhaps certain salts in the thin segments lined by the clear, pavement epithelium the extent of this absorption must differ widely in tubular segments having this structure, and that, therefore, urine of different degrees of concentration must as a consequence enter the collecting ducts.

58 (583)

On fundamental changes in the action of some alkaloids upon frogs after cardiectomy or ligation of one aorta.

By **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

At the last meeting of the American Physiological Society I reported that in cardiectomized frogs an injection of adrenalin will still cause dilatation of the pupil and an injection of strychnine will cause a tetanus. At the February meeting of this society I reported on the action of morphin on cardiectomized frogs. An injection of 10 or 15 mgr. of morphin in living, normal, medium-sized frogs causes at first no perceptible effect; after a few days a tetanus may set in, while there is at no time a depression. In cardiectomized frogs, however, an injection of 6 or 8 mgr. of morphin causes the appearance of tetanic symptoms in less than an hour. When 10 to 20 mgr. are injected the tetanus is preceded by a paretic state. After an injection of 25 to 30 mgr. the animal becomes paralyzed very early, to be interrupted later by only short weak tetanic movements.

I have since found that also after the ligation of one aorta, especially of the right one, a tetanus will very frequently develop two or three hours after an injection of 10 or 15 mgr. of morphin; in some cases the tetanus may persist as long as twenty hours. In some cases two or three hours after the development of the spasmodic state the tetanus became temporarily interrupted by paralysis which lasted only 30 to 60 minutes.

Since the beginning of April it often became difficult to obtain in cardiectomized frogs a tetanus; even with small doses of morphin the effect was in many cases complete paralysis, which set in very early after the injection. The same was true also of frogs with one aorta ligated. Here the heart might be seen beating forcefully for hours while the animal is completely paralyzed. The fact reminds one of the behavior of nerve and muscle irritability in spring frogs.

Finally in a small percentage of cardiectomized frogs curare

(curarin) was found to produce tetanic symptoms. It was found further that when the right aorta has been ligated and the tissues of one leg or of both were firmly contracted, under the exclusion of the sciatic nerves (Claude Bernard), curarin produced in most cases a stage of definitely increased reflexes and even of short tetanic attacks.

59 (584)

On the convulsant action of acid fuchsin (Abel and Barbour) in cardiectomized frogs.

By **DON R. JOSEPH** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In experiments carried out in this laboratory it was shown that in the absence of the cardiovascular mechanism alkaloidal solutions are well distributed through the body; that, for instance, injections of adrenalin cause dilatation of the pupil and injections of strychnine cause the development of spasms. It was further found that the action of some substances may be even greatly accelerated and more effective in cardiectomized than in normal frogs. Morphine, for instance, may cause a tetanus in 40 to 50 minutes.

In a recent communication of Abel and Barbour it was reported that an injection of a comparatively large dose (one mgr. and more per grm. body-weight) of acid fuchsin into a frog may cause after many hours (even as much as 20) the appearance of a series of convulsions. These investigators discovered further that in frogs in which the anterior third of the cerebral lobes was removed, such convulsions may appear very soon, 13 minutes and less, after an injection of only a small dose of the fuchsin, *e. g.*, 0.35 mgr. per grm. body-weight. With the consent of Professor Abel we investigated the behavior of fuchsin in cardiectomized frogs in which the brain remained intact. The following is a brief preliminary report of the results.

Observations were made so far on about forty frogs. In all cases in which fuchsin was injected into the dorsal lymph sac of cardiectomized frogs, convulsions never failed to appear and the time of appearance was never longer than half an hour after the

injection. In 27 frogs the injected dose of fuchsin was less than 0.1 mgr. per grm. of body-weight. In eighteen frogs the dose was 0.05 mgm. per gram body-weight, the time of onset of convulsions varying between 4 and 15 minutes. In some of these cases the entire dose for the frog amounted to less than one milligram of the fuchsin. In a few frogs the effective dose was not more than 0.025 mgm. per gram body-weight.

We have here another instance in which the action of a substance is greatly accelerated and much more effective in animals without a circulation than in normal animals. The experiments seem to show further that the minimum toxic dose of fuchsin is for cardiectomized frogs much smaller than even for frogs with the anterior part of the brain removed.

60 (585)

On the presence of dextrose in the exudate of pulmonary edema.

By **I. S. KLEINER** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Injection of adrenalin causes glycosuria and hyperglycemia. In some instances the animals which receive adrenalin die of pulmonary edema. The chemical composition of the exudate of pulmonary edema has never been investigated. An examination of the exudate of rabbits which died from pulmonary edema after receiving adrenalin revealed the presence of a considerable amount of dextrose. The causation of pulmonary edema by the injection of adrenalin is, however, a matter of mere accident and cannot be relied upon in a systematic study. After various attempts we found that inhalation of ammonia can be fairly well relied upon to produce edema and produce it in a quantity sufficient to make a quantitative test for a reducing substance. The exudate did not clot, which shows that no pure blood was mixed with it. The number of experiments, although not yet large, permits a definite preliminary report. Besides analyzing the pulmonary exudate, in most cases a quantitative analysis of the blood for reducing substances was made and in some instances also of the urine. Pulmonary edema was also produced in two normal ani-

mals and in one animal which received an intravenous infusion of dextrose. The results may be briefly summarized as follows: The exudate of pulmonary edema contains dextrose or a reducing substance. The concentration seems to be in general equal to that of the blood. Two hours or longer after an intramuscular injection of adrenalin the exudate of the pulmonary edema may contain 0.5 per cent. and more of dextrose, a quantity, which, at least so far, usually slightly exceeded that of the corresponding blood.

61 (586)

The effect of culture medium contaminated with the excretion products of Paramæcium on its rate of reproduction.

By **LORANDE LOSS WOODRUFF.**

[*From the Sheffield Biological Laboratory of Yale University.*]

A summary is presented of the initial experiments of a series which is planned to elucidate, if possible, some of the complex factors at work in a "hay infusion"; for example, such as those which determine the inter-dependence of the organisms, their sequence, time of appearance, disappearance, etc. The data outlined were derived from the study of: (*a*) the effect of different volumes (2, 5, 20 and 40 drops) of culture medium on the rate of reproduction of *Paramaecium;* (*b*) the effect of changing the culture medium daily and on alternate days on the rate of reproduction of *Paramaecium;* and (*c*) the effect of culture medium, in which large numbers of paramæcia have been living, on the rate of reproduction of *Paramaecium.* It is believed that the results obtained justify the following conclusions:

1. The rate of reproduction of *Paramaceium aurelia* and *Paramaecium caudatum* is influenced by the volume of the culture medium, within the limits tested, and the greater the volume the more rapid is the rate of division.

2. Paramæcia excrete substances which are toxic to themselves when present in their environment, and these substances are more effective when the organisms are confined in limited volumes of culture fluid.

3. The excretion products of paramæcia play an appreciable part in determining the period of maximum numbers, rate of decline, etc., of this animal in "hay infusions."

62 (587)

On the origin of glycocoll in the animal body.

By **A. I. Ringer.**

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

The various forms of protein, with the exception of casein and gelatin, contain about four per cent. of their N in the form of preformed glycocoll N. On giving benzoic acid or its salts to animals, they have the power of detoxicating it by combining it with glycocoll and forming hippuric acid.

About eleven years ago, Parker and Lusk employed this fact in an attempt to determine the maximum amount of hippuric acid that rabbits can produce. They studied the relationship between the total N in the urine and the glycocoll N eliminated as hippuric acid, HN/N. They found that for every 100 grams of total N excreted in the urine, an average of about 4 grams of N was eliminated in the glycocoll radical of the hippuric acid. After administering the first large dose of lithium benzoate to the animals, they invariably obtained a much higher HN/N ratio, 9.01 per cent. in experiment III., 7.14 per cent. in experiment IV., and 7.87 per cent. in experiment VI. They regarded this as a "sweeping out" of surplus glycocoll.

Since then, Wiechowski and Magnus-Levy have studied the same problem. Wiechowski obtained HN/N ratios of 45.4, 55, 50 and in one case, even as much as 64 per cent. Magnus-Levy found in his rabbits a maximum ratio of 25 and 28 per cent., in his sheep 27.8 per cent.

To investigate the cause of these discrepancies, Professor Lusk kindly suggested that I continue the study of this problem.

Experiment I.

A goat weighing 42.3 kg. was employed. In Table I., the results of this experiment are summarized. It shows that the animal was able to eliminate in its urine a good deal more glycocoll than is found preformed in the proteins of its tissues. The hippuric acid formation, *i. e.*, the glycocoll elimination does not depend upon the amount of protein catabolized, but within certain

TABLE I.

Date, 1910.	Period.	Weight.	Total N, Gr.	Hippuric Acid, Gr.	Hippuric Acid Nitrogen, Gr.	Hippuric Acid N % / Total N	A Benzoic Acid Fed.	B Benzoic Acid Excreted as Hippuric Acid.	A/B Per Cent.	Hippuric Acid per Kg. Weight.	Remarks.
February 25	I.	——	5.441	——	——	——	——	——	——	——	——
February 26	II.	——	6.39	——	——	——	——	——	——	——	——
February 27	III.	——	5.027	——	——	——	——	——	——	——	——
February 28	I.	42.3	4.303	10.19	0.805	18.7	8.47	6.95	82.04	0.247	250 gm. hay, 100 gm. oats.
March 1	II.	——	Urine Lost			23.06	16.94	——	——	——	88 gm. hay, 195 gm. white bread, 190 gm. milk.
March 2	III.	41.25	7.23	19.46	1.537	21.26	16.94	13.27	78.32	——	200 gm. white bread, 150 gm. hay.
March 3	IV.	——	9.08	27.75	2.193	24.14	21.18	18.93	89.39	——	100 gm. bread, 100 gm. cabbage, 160 gm. carrots.
March 4	V.	40.1	6.45	11.06	0.874	13.54	8.47	7.54	89.06	——	Ate what was left from previous day, no more.
March 5	VI.	——	6.36	30.93	2.443	38.41	25.42	21.10	83.05	0.786	Starving.
March 6	VII.	39.35	8.06	28.28	2.234	27.72	25.42	19.03	74.86	——	Starving.
March 7	VIII.	——	7.81	15.59	1.230	15.77	25.42	10.63	41.83	——	Starving. All benzoate one dose.
					Intermission of sixteen days.						
March 23	IX.	——	8.12	19.96	1.577	19.41	42.36	13.61	32.15	——	Starving.

limits runs parallel to the amount of benzoic acid fed. In period VI., it reached its maximum. 38.4 per cent. of the total N was eliminated as glycocoll N in the hippuric acid.

Where do these enormous quantities of glycocoll originate? It might be argued that the source of the glycocoll found in the system lies in the different vegetable foods which are known to contain rather large quantities of free amino-bodies. But after giving 8 grams of sodium benzoate to a calf two weeks old which had had no other form of food but milk, the protein of which contains no glycocoll, it was found that it was able to form and eliminate hippuric acid glycocoll as readily as the adult goat.

Ingestion of glycolic or glyoxylic acid with very large quantities of sodium benzoate in rabbits, does not increase the quantity of glycocoll formed as indicated by the amount of hippuric acid eliminated. This might have been possible from their chemical relationship to glycocoll. (A detailed account of these experiments will be given in the final report.)

On careful consideration of the nitrogen metabolism in animals during the benzoate period, a possible explanation of the origin of glycocoll suggests itself. The goat during the three days of the foreperiod, excreted an average of 5.6 grams of N. Excepting the first day, which may be due to a sudden change in the quantity of food, there is a marked rise in the protein metabolism throughout the course of the benzoate period. This was observed in all the experiments and in all the varieties of animals that have been experimented upon. Furthermore, the increase in the protein destruction, *i. e.*, that increase in the N elimination above the normal, or above a previous day of a smaller benzoate dose, is always two to three times greater than the amount of nitrogen that has been eliminated as glycocoll in the hippuric acid molecule.

In Table II., the results of experiments on two rabbits are recorded. These show that the amount of urea N plus ammonia N during the benzoate period does not differ from normal days, and also that the extra N catabolized is much greater than the amount of N that was eliminated in the form of hippuric acid.

All these facts suggest the possibility that the glycocoll excreted as hippuric acid does not come from the fraction of protein that would have been metabolized had no benzoate been given,

but entirely from the extra protein which is destroyed, due to the presence of the toxic substance. We cannot state with any degree of certainty, what the character of the intermediary processes is, but that it is specific and peculiar seems very probable, for none of the extra N destroyed goes over into urea. It is all eliminated either as glycocoll or as undetermined N.

TABLE II.

Rabbit No. 6.

Date, 1911.	Period.	Weight.	Total N.	Urea N.	Per Cent. of Total N.	NH_3N.	Per Cent. of Total N.	Hippuric Acid, N.	Per Cent. of Total N.	Benzoic Acid Fed.
March 12	I.	1.8	0.802	0.7024	87.6	0.232	2.89	—	—	—
March 13	II.	1.72	1.147	0.796	69.4	0.36	3.177	0.088	7.7	1.7
March 14	III.	1.60	0.913	0.782	85.67	0.166	1.82	—	—	—

Rabbit No. 7.

March 23	I.[1]	1.96	0.596	0.487	81.68	0.043	7.23	—	—	—
March 24	II.	1.71	1.369	0.9673	70.66	0.1127	8.23	0.082	6.0	1.7
March 25	III.	1.68	1.199	1.0898[2]						

63 (588)

Experiments on the diffusibility of alkaloids through rubber.[3]

By **J. B. SIDBURY** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

Rosenbloom and Gies have found that various ether-soluble substances, when dissolved in ether and placed in rubber bags immersed in ether, readily pass through the rubber membranes

[1] Period of about 12 hours.

[2] Urea + NH_3N = 90.9 per cent.

[3] This study is one of a projected series on *physico-chemical conditions in the cell*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

thus imposed.[1] We have found that various alkaloids and some related substances readily diffuse through rubber under such conditions.

Our experiments were conducted as follows: A moderate quantity of the pure ether-soluble substance was mixed with 15–25 c.c. of ether.[2] This mixture was poured through a funnel into a new air-tight rubber condom in such a way as to preclude the possibility of overflow upon the external surface. The bag was then immersed in about 50 c.c. of ether in a narrow salt-mouth bottle 7 inches high. With the bag suspended at full extension in this position, its mouth was about an inch above the opening in the bottle. The protruding condom was supported in the neck of the bottle by a tightly fitting cork stopper, which also served to keep the bag closed. After a diffusion period of convenient length (sometimes 2 to 5 days),[3] the condom was removed from the bottle, the ether diffusate was poured into a porcelain dish, and the ether completely removed by evaporation on a steam bath. At least one appropriate test was then applied to the residue.[4]

Meanwhile, the ether solution in the condom was removed. A large volume of water was then poured into the suspended bag, which, during its distention by the water, was carefully examined for signs of leakage. In a few instances defective membranes temporarily rendered the outcome doubtful. All results with such bags were ignored, of course. Each of the tests, even after reliable positive responses, was repeated at least once with a *new* rubber bag.

The substances named below (the complete list of those already tested in this connection) are readily diffusible under the conditions of these experiments:

A. Apomorphin, atropin, brucin, caffein, cocain, codein, colchicin, coniin, morphin, narcein, narcotin, nicotin, physostigmin, quinin, strychnin, veratrin.

[1] Rosenbloom and Gies, *Journal of Biological Chemistry*, 1911, ix; *Proceedings of the American Society of Biological Chemists*, p. xiv (December, 1910); also, PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1911, viii, p. 71.

[2] Substances which did not dissolve readily were triturated with ether in a mortar.

[3] Some of the alkaloids pass through rubber almost immediately under the conditions of these experiments.

[4] In the experiments with nicotin, the "tobacco odor" of the concentrated liquids was very pronounced.

B. Acetanilid, antipyrin, phenacetin, picric acid, picrotoxin, pyramidon, salicylic acid.

Experiments with other solvents, and with additional substances of alkaloidal type, will be added to this series.

64 (589)

Notes on Fischer's theory of the influence of acids in the production of edema.[1]

By **F. G. GOODRIDGE** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

Several years ago Gies published some of the results of a preliminary study of the effects of acids on tendon collagen.[2] Last December Kantor and Gies reported their observation that collagen fibers from tendon immediately swell markedly in *free* acid but do not swell at all in any strength of *combined* acid[3]—facts on which they base a new microscopic test for free acid. These results naturally led Kantor and Gies to consider the relation of such facts to Fischer's theory of edema, which they were investigating at the time these observations were made. Lately we have gone into this particular phase of the matter with some experiments on fibrin. Similar experiments are under way with other colloids and with various tissues.

Fischer's general conclusion in regard to edema is stated in the following terms:[4]

"A state of œdema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water in increased above that which we are pleased to call normal. The accumulation of acids within the tissues,

[1] This study is one of a projected series on *proteins and their combining qualities*. which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

[2] Gies, *Science*, 1907, xxv, p. 462.

[3] Kantor and Gies, *Journal of Biological Chemistry*, 1911, ix; *Proceedings of the American Society of Biological Chemists*, p. xvii.

[4] Fischer, "Edema — a study of the physiology and the pathology of water absorption by the living organism," 1909, p. 99.

brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, *though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids or through the conversion of colloids having but little affinity for water into such as have a greater affinity must also be borne in mind.*" [1]

Fischer's views on the influence of acid in the production of pathological edemas appear to us to be over-emphasized. Special stress was laid by Fischer upon lactic acid as a causative factor in pathological edema, but apparently no experiments were performed with that acid. The action of electrolytes on the power of lactic acid to excite water absorption by colloids was not ascertained. Acids such as hydrochloric were Fischer's chief reliance in his experiments with acids.

We find, when moist shreds of fibrin are severally suspended in gelatin solution, peptone solution, fresh egg white, blood, milk, and meat juice, that hydrochloric acid solution (0.2 per cent. to 10 per cent.) may be added to the mixture in each case *in any proportion* without inducing visible effects on the fibrin shreds, *unless sufficient acid is added to provide an excess in the* FREE *state.* Very large quantities of acid may be added to such mixtures without appreciable bloating effect.[2] If the colloids in the artificial solutions and protoplasmic liquids enumerated above are combined with any proportion of the acid up to exactly their *maximum* affinity for it (hydrochloric acid), so that the liquids while strongly acid to litmus respond negatively to tests for *free* acid, then moist fibrin shreds can be kept in such acid fluids indefinitely without swelling to any perceptible degree. Warm concentrated gelatin solutions may be put into these conditions of free and combined acidity. After such solutions have been permitted to gelatinize, moist fibrin shreds which have been imbedded in the resultant jellies swell perceptibly, provided the gelatinized mass contains *free* acid, *but the shreds do not appear to absorb water from the medium if its contained acid is only in* COMBINED *form.* It is obvious that such

[1] Special emphasis is laid by us on the part of the quotation which we have italicized.

[2] Similar observations have been made with alkaline mixtures. We expect to describe, in the near future, a new test for free alkali based upon the behavior of fibrin in association with dissolved protein in alkaline media.

facts have an important bearing on any theory of acid causation of edema.

Our experiments do not permit us to deny that acids may be influential factors in the causation of edematous processes. Our results emphasize the fact, however, that the acids which may be produced in, or that are carried into, tissues tend to unite there with non-colloidal basic radicals and with dissolved colloids before combining with suspended colloids. The chemical means and excretory processes by which *living* protoplasm maintains a state of reaction-constancy cannot easily be overcome. In Fischer's published experiments on the bloating effects of acids, *large* excesses of *free* acid were present in all but a few cases. Would Fischer contend that edematous tissues contain *free* acid?

We feel that acids are not the only causes of colloidal water absorption in edema. Results obtained by Berg and Gies[1] several years ago indicate that *enzymes* facilitate any such influence that acids, whether free or combined, may exert; and vice versa. Fischer himself alludes, "in passing" (p. 109), to a result in harmony with that view. The italicized portion of the foregoing quotation from Fischer's book is broad enough to include enzyme influences and all other contributory factors. Experiments along these lines are still in progress.

65 (590)

The relation of the toxic dose of horse serum to the protective dose of atropin in anaphylaxis.

By **HOWARD T. KARSNER** and **JOHN B. NUTT.**

[*From the McManes Laboratory of Pathology, University of Pennsylvania.*]

This study was prompted by the publications of Auer and Lewis,[2] and of Auer,[3] which definitely demonstrated the prophylactic action of atropin sulphat in the asphyxia of immediate anaphylaxis. The results of these writers have been confirmed repeatedly.

[1] Berg and Gies, *Journal of Biological Chemistry*, 1907, ii, pp. 508 and 522.

[2] Auer and Lewis, *Jour. A. M. A.*, 1909, viii, 458; *Jour. Exp. Med.*, 1910, xii, 153; *ibid.*, p. 165.

[3] Auer, *Amer. Jour. of Physiology*, 1910, xxvi, 439.

In our own studies we used guinea pigs averaging about 400 grams in weight and sensitized by subcutaneous administration of 0.05 c.c. horse serum. The atropin was injected intravenously five minutes before the toxic dose of horse serum which also was administered into the jugular vein.

The study shows that as the toxic dose of horse serum is increased the protecting dose of atropin must also be increased, but the increase in protecting dose is not proportionate to that of the horse serum. The curve of protecting dose rises much more sharply than that of horse serum and finally a point is reached where the animal succumbs to the dose of atropin. A 400-gram guinea pig is killed almost instantly by a dose of 0.060 gram atropin.

That the effect of the atropin is physiological and not due to any alkaloidal combination with the toxic fraction of the horse serum is shown by the fact that a mixture of atropin and horse serum incubated at 37° C. and dialyzed for four days killed sensitized animals whereas a control in the same proportions but not dialyzed saved the animals from anaphylactic death.

66 (591)

The effect of specific vaccines in the typhoid of rats and mice.

By **EDNA STEINHARDT** and **THOMAS FLOURNOY.**

[*From the Laboratory of Hygiene, University of Michigan, and the Pathological Laboratory, Bellevue Hospital.*]

Owing to the impossibility of infecting small laboratory animals by feeding with typhoid bacilli, the immunity produced by specific vaccines has always been tested by subcutaneous or intra-peritoneal inoculations of the living culture.

These methods do not produce a disease comparable to human typhoid, but when rats and mice are fed with certain of the paratyphoid group they contract a disease whose pathology does closely resemble it; therefore these were used for the comparative study of the vaccines. The Danysz virus (one of the Gaertner group) was the test organism.

White mice were used in the first series and the vaccines tested were killed cultures, Vaughan's residue, sensitized bacilli (Bes-

redka's vaccine) and protective inoculations of serum, all the injections being given subcutaneously to make them more comparable with those in man. When later, these vaccinated mice were fed with living cultures, no protection was shown, sickness usually fatal occurred exactly as in the untreated cases. After an interval, all of the mice that recovered were re-fed with the living culture and all contracted the disease again, showing that no immunity had been established.

Rats being less susceptible than mice, the experiments were repeated upon them. The vaccinated animals when fed with living cultures were not protected but those which had been previously fed with small doses of the living virus, were completely immune to subsequent feeding with large doses. Experiments are now being carried out to see if these rats are also immune against intra-peritoneal and sub-cutaneous inoculations and if it is a strictly specific immunity.

Experiments on treatment, specific and otherwise, gave negative result in mice; in rats the investigations are still being carried on.

67 (592)

The relationship of the left suprarenal capsule to the sugar production of the liver in dogs.

By **J. J. R. MACLEOD, C. D. CHRISTIE** and **R. G. PEARCE.**

[*From the Physiological Laboratory, Western Reserve University, Cleveland, O.*]

We have obtained certain results bearing on the relationship between the left suprarenal capsule and the left splanchnic nerve-control of the glycogenic function of the liver which, in view of the topical interest in this subject, we believe it well to report at present, especially since it will take some considerable time to complete the investigation of which they form a part. The percentage amount of reducing substance in the blood of the inferior vena cava, opposite the hepatic veins, was determined by Reid's method at varying periods after stimulation of the left splanchnic nerve. In the first series of results given in the table the left suprarenal capsule was intact; in the second series this gland was excised.

TABLE.

PER CENT. REDUCING SUBSTANCE IN BLOOD OF VENA CAVA INFERIOR OPPOSITE ENTRANCE OF HEPATIC VEINS IN DOGS FED WITH EXCESS OF CARBOHYDRATE.

A. *Without Excision of Suprarenal.*

No. of Expt.	Before Stimulating.	During Stimulation.	After stimulation Removed.	Remarks.
16	0.136	0.176 (7 min.)[1]	0.184 (10 min.)[2]	
18	0.124	0.110 (7 min.)	0.144 (30 min.)	No rise in
19	0.130	0.176 (10 min.)	0.148 (30 min.)	blood pres-
20	0.199	0.265 (5 min.)	0.415 (30 min.)	sure.
21	0.151	0.195 (7 min.)	0.168 (23 min.)	
24	0.132 0.121 (30 min. later)	0.154 (7 min.)	——	
25	0.194 0.213 (30 min. later)	0.280 (5 min.)	——	
32	0.111 0.122 (30 min. later)	0.166 (9 min.)	——	
	B. *After Removal of Left Suprarenal Capsule.*			
27	0.118	0.124 (10 min.) 0.109 (25 min.)	——	
28	0.120 0.082 (30 min. later)	0.101 (5 min.) 0.103 (20 min.)	——	
29	0.171 0.173 (30 min. later)	0.177 (16 min.)	——	
30	0.163 0.127 (30 min. later)	0.113 (15 min.)	——	
31	0.249 0.248 (25 min. later)	0.270 (5 min.) 0.256 (20 min.)	——	

In every case, but one (no. 18) in which the suprarenal was intact there was a distinct increase in the reducing power of the blood, but when the suprarenal was exsected no increase occurred (except a slight transitory increase in no. 31). The blood pressure rose in all cases except no. 18.

68 (593)

Comparison between the blood flow in the arm and in the hand.

By **A. W. HEWLETT** and **J. G. VAN ZWALUWENBURG.**

[*From the Department of Internal Medicine, University of Michigan.*]

In 1909 the authors published a plethysmograph method for determining the rate of blood flow in the arm (Heart, I., 87) The results obtained by this method for normal individuals, partly

[1] The time periods in brackets indicate when the specimens of blood were removed in (1) after the beginning of stimulation; in (2) after the stimulation had been discontinued.

or completely stripped to the waist, under the ordinary conditions of room temperature, usually lay between 2 and 5 c.c. of blood flow per 100 c.c. of arm substance per minute. More recently, by a calorimeter method, G. N. Stewart has obtained rates in the hand approximately 10 c.c. of blood flow per 100 c.c. of hand substance per minute. We have attempted to compare the two methods because it seemed improbable to us that this discrepancy could depend entirely upon variations in external conditions, such as the room temperatures or the amounts of clothing worn.

The plethysmograph method was applied to the hand and the results compared with those obtained by the calorimeter method on the hand and with those obtained when the plethysmograph included the forearm and the lower arm. Our method does not give very satisfactory results for the hand. The curves are apt to rise abruptly when the pressure is applied and the rate of swelling remains constant but a short time before it lessens, owing apparently to the small venous capacity of the hand.

It is nearly always possible, however, to obtain curves where the rate of inflow is nearly constant for 2 c.c. or more. By selecting such parts of the tracings, an approximate rate of flow in the hand was obtained.

TABLE I.

	Average Room Temp.	Average Calorimeter Temp.	Rate by Calorimeter.	Plethysmograph Plate.			Remarks.
				Hand.	1st Arm.	2d Arm.	
Co.	24°	25.7°	3.1	4.8	2.9	3.1	Thick skin, hand cold.
Ca.	23°	29.8°	4.8	7.8	2.3	2.4	Hand cool.
So.	27.5°	29.0°	10.0	10.2	4.5	4.5	Hand warm.

In the experiments charted in Table I. the individuals were stripped to the waist. The rate of flow in the hand was first determined by Stewart's method. After this the hand was dried and successive determinations of the blood flow, by the plethysmograph, were made on the forearm, the hand, and again on the forearm. A glance at this table indicates that the blood flow in the hand was relatively faster than it was in the combined hand, forearm, and lower arm; and, furthermore, that the plethysmograph method, when applied to the hand, gave somewhat faster rates of flow than did the calorimeter method. In these experiments the plethysmograph contained air only.

In order to compare the two methods more closely a second series of experiments were performed in which the individual remained clothed and the hand, or arm, in the plethysmograph was covered with water of the same temperature as the water that had been in the calorimeter. The results of these experiments, shown in Table II., agree with the first set.

TABLE II.

	Average Room Temp.	Average Calorimeter.	Rate by Calorimeter.	Plethysmograph Rate.	
				Hand.	Arm.
V.Z.	24.5°	29.8°	7.4	9.6	
V.Z.	24°	28.0°	7.4	8.3	
V.Z.	25.5°	29.6°	4.7	8.6	3.1

These observations indicate that the blood flow in the hand, relative to its volume, is faster than is the flow in the combined forearm and hand. We found considerable differences in the results obtained by the two methods used on the hand. We do not know the exact causes of these differences but are inclined to believe that both hand methods are subject to greater error than is the plethysmograph method when applied to the arm.

69 (594)

On the phagocytic inclusion of carmine particles by sarcoma cells growing in vitro with consequent staining of the cell granules.

By **F. M. HANES** and **R. A. LAMBERT**.

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.*]

The cells of rat and mouse sarcomata when cultivated in vitro show active amœboid movements and wander for considerable distances into the surrounding plasma. Upon adding finely powdered carmine particles to the plasma medium the wandering tumor cells take up the particles in an active phagocytic manner. The carmine particles within the cells are easily distinguishable by their opacity and angularity. The carmine is partially dissolved within the cell and brings into evidence the granules of the

cytoplasm. These granules are round, of very constant size and are stained pink by the carmine. They occupy the entire cytoplasm. By means of Altmann's fixative and stain exactly similar granular pictures are obtained.

70 (595)

The formation of metastases after an intravascular injection of tumor emulsions.

By **I. LEVIN** and **M. J. SITTENFIELD.**

[*From the Department of Pathology of Columbia University, George Crocker Special Research Fund, New York.*]

It is generally accepted that metastasis in malignant tumors is formed by the proliferation of tumor cells which have been transported to distant parts of the organism through the blood or lymph channels. The cells of the primary tumor penetrate in some manner into the lumen of the vessels, are swept away by currents as emboli, and finding lodgment in some distant part of the organism, they proliferate and form secondary tumors.

This conception of the formation of metastasis was established through observation of autopsy material and no direct experimental proof of the matter was adduced up to the present. All experiments with intravascular injection of human tumor material into animals either gave negative results or were entirely untrustworthy.

In all the extensive literature of the last decade on the subject of the transplantable cancer of the white mouse and rat there appears no statement in regard to intravascular injection of tumor material with the aim of forming metastasis. The only exception is a short note by Graf, who obtained negative results.

Metastasis in malignant tumors of the white mouse and rat occurs rarely as compared with human cancer, and the channels for the transportation of the tumor cells are in a majority of cases the blood vessels. The reason for the rare occurrence of metastasis in the rat and mouse Ehrlich, in accordance with his athreptic theory of immunity, sees in the fact that tumors in these animals are usually of extreme malignancy and grow to very large

size. The cells of the primary tumor use up all the specific food found in the organism of the host, and the cells transported from the primary tumor to other regions of the organism do not find the necessary nourishment and consequently can not proliferate. Carl Lewin on the other hand thinks that the fact that metastasis in these animals takes place only through the aid of the blood vessels and not the lymphatics, accounts for the rare occurrence of metastasis in these animals. As was shown by M. B. Schmidt through his observations on human cancer, blood is capable in a majority of cases of destroying cancer cells, found within the blood vessels.

The study of the influence of the blood upon cancer cells carried by it and the capacity of such cells to form metastasis may serve to clear up a number of phenomena in the genesis of cancer. In view of this a systematic study was undertaken by the writers on the experimental formation of metastasis, of which the present communication forms the first report.

The experiments consisted in an injection of a tumor emulsion into the jugular vein or carotid artery of a white rat, and were conducted on three different tumors.

Sarcoma of the White Rat.—This tumor is very malignant, grows to a large size, and the occurrence of metastasis after a subcutaneous inoculation is very rare. In hundreds of animals inoculated by the writers local dissemination was observed not more than in a half dozen cases, and once only was metastasis found in the liver. An emulsion of this tumor was prepared by cutting it in Haaland's mincing machine, grinding in normal salt solution and filtering through a layer of coarse gauze. The milky opalescent fluid contains a sufficient number of living cells to produce a tumor growth after a subcutaneous inoculation. Thirty-six rats received an injection of this emulsion into the jugular vein. The animals were killed at periods ranging from eight days to four weeks after the injection and a thorough search made in all the organs for metastasis. Not in a single instance was a metastatic tumor found. No microscopical study was made of the organs which appeared normal on gross inspection, since this investigation does not concern itself with the question, where the tumor cells circulating in the blood find lodgment, but whether such a

cell transported into a certain organ will form there a visible metastasis. All suspicious nodules found anywhere and all lungs appearing abnormal on gross inspection were examined microscopically with negative results. The same negative results were obtained in six rats, where the injection was made into the carotid artery, and the animals survived. This method is very difficult of execution and the animals usually die a few minutes after the injection from respiratory paralysis, while the heart continues its action a few minutes longer.

Carcinoma of the White Rat (Flexner-Jobling).—This tumor is not as malignant as the previous one, but metastasis occurs frequently after a subcutaneous inoculation. The metastasis is usually found in the lungs, but it was also observed by Flexner and Jobling in the kidney, the heart and even in the lymphatic glands, though the authors believe that in most cases the metastases were produced through the blood current. Sixteen rats received an injection of an emulsion of this tumor into the jugular vein. In three animals metastasis was found in the lungs, no other organ showed any metastasis. Six rats survived an injection into the carotid and of these animals one showed metastasis in the lungs. In another rat, in which the injection was made in the carotid against the stream of blood, the animal was found dead twelve days later. At the autopsy a nodule was found on the wall of the left ventricle. The animal remained dead in the cage over night and the specimen had greatly deteriorated, still the nodule resembled microscopically the picture described by Flexner and Jobling of a metastasis found by them in the heart.

Sarcoma of the White Mouse.—Ehrlich has shown, that when this tumor is inoculated subcutaneously into a rat, there forms a small nodule, which remains for 8–10 days, and is then absorbed. No metastasis formation of this mouse tumor into a rat was ever noted. An emulsion of this tumor was injected into the jugular vein of twelve rats. The rats were killed in periods of 4–8 days and in two of the animals metastatic nodules were found in the liver. Neither in the lungs nor in any other organ was there found any metastasis.

The results of these experiments seem to indicate in accordance with the opinion of M. B. Schmidt, that cancer cells introduced

into the blood circulation in a majority of cases lose their proliferating power. Ehrlich's opinion that the most virulent tumors do not form metastasis also seems to be correct, as no metastasis was formed after the injection of the most virulent sarcoma of the rat. But the athreptic theory does not explain this fact. None of these animals had any sarcoma growth anywhere and consequently the sarcoma cells introduced into the circulation could find all the necessary specific food.

The most interesting phenomenon observed in the course of these experiments consists in the fact, that while the Flexner tumor found lodgment in the lungs after an intravascular injection, the mouse sarcoma produced metastasis only in the liver. It would seem that the different topographic distribution of metastasis in the different kinds of malignant tumors is due not so much to the difference in the channels through which the cancer cells are transported, and which were identical in all experiments reported here, but to a specific affinity between cancer cells and cells of certain organs. The comprehension of this specific affinity between a cancer cell and a particular part of the organism of the host may be helpful in elucidating many factors in the genesis of tumors and will be the main object of the further study of the experimental metastasis formation by the writers.

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Members elected at the forty-third meeting:

Alfred E. Cohen, T. H. Montgomery.

Dates of the next two regular meetings:

May 17, 1911. October 18, 1911.

PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

FORTY-FOURTH MEETING

UNIVERSITY AND BELLEVUE HOSPITAL
MEDICAL COLLEGE

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CONTENTS.

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SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty fourth meeting.

University and Bellevue Hospital Medical College. May 17, 1911.

71 (596)

The balance of acid-forming and base-forming elements in foods, and its relation to ammonia metabolism.

By **H. C. SHERMAN** and **A. O. GETTLER.**

[*From the Laboratory of Food Chemistry, Columbia University.*]

In continuation of previous work[1] ash analyses have been made of a number of foods and from the percentages of total sulphur, phosphorus and chlorine on the one hand, and of sodium, potasium, calcium and magnesium on the other, the excess of acid over base or of base over acid which will result from the oxidation of the food has been calculated. Previous ash analyses have also been studied and supplemented by such determinations as were necessary to permit the calculation of this balance for a wide range of food materials. Meats and eggs show a predominance of acid-forming elements; in fruits and vegetables the base-forming elements predominate. From this standpoint the fruits and vegetables tend to balance the meats of the diet. Milk and the cereals contain acid-forming and base-forming elements in more nearly equivalent proportions.

Through the kindness of Mr. L. H. Smith, samples of corn which had been bred through ten generations for high and low protein content respectively were obtained from the Illinois Agricultural Experiment Station. The ash-analyses of these were very similar except for the higher sulphur content of the high protein corn, which resulted in this sample showing a slight predominance of acid-forming elements, while in the low protein corn the base-forming elements predominated.

In order to determine to what extent the excess of acid brought

[1] Sherman and Sinclair, *Jour. Biol. Chem.*, Vol. III., 307.

into metabolism by the oxidation of the food is neutralized by ammonia in man, an experiment was made in which the influence upon ammonia excretion of a known change in the diet was studied quantitatively. The change of food consisted in substituting rice for potatoes in a simple mixed diet and (neglecting the feces but allowing for the unoxidized sulphur excreted during each period) was calculated as equivalent to the introduction of 28.3 c.c. normal acid per day. The increased ammonia excretion was equivalent to 10.7 c.c. normal acid per day. Thus, only about one third of the extra acid introduced by the change of food was eliminated as ammonia salt.

The authors take pleasure in acknowledging their indebtedness to Professor Mandel for the privilege of carrying on a part of the work in his laboratory at the University and Bellevue Hospital Medical College.

72 (597)

The determination of small amounts of iodine in organic combination — A modification of Hunter's method.

By **E. C. KENDALL.**

[*From the Pathological Department of St. Luke's Hospital, New York, F. C. Wood, Director.*]

Hunter's method for the determination of iodine in organic combination consists of fusion of the organic matter, and the formation of sodium or potassium iodide. The iodide is oxidized to iodate with sodium hypochlorite, phosphoric acid is added, and the excess of chlorine is removed from solution by boiling. Potassium iodide is now added and each molecule of iodate liberates six atoms of iodine. The liberated iodine is titrated with sodium thiosulphate, the final reading being six times the amount of iodine originally present.

The removal of chlorine by boiling is a time-consuming and uncertain operation. The writer has modified the above method by removing the excess of chlorine from solution with phenol. The free chlorine adds directly to the benzol ring forming an unionized compound which does not interfere with subsequent operations. This modification makes the above method accurate and rapid.

73 (598)

Beef extract as a "constant" culture medium for Paramæcium aurelia.

By **LORANDE LOSS WOODRUFF** and **GEORGE ALFRED BAITSELL.**

[*From the Sheffield Biological Laboratory of Yale University.*]

Previous work with pedigree cultures of *Paramœcium aurelia* and *caudatum* has apparently shown that the life history of these forms, when bred continuously on infusions of hay made up exactly the same from day to day, tends to run in a cycle which terminates with the death of the culture. Previous work (Woodruff) has also shown that *Paramœcium aurelia* may be bred indefinitely on a culture medium which is frequently varied.

In view of these results the following question is of interest: Is the longevity of paramæcia on a "varied environment" dependent upon the intrinsic stimuli from the frequent changes in the medium, or is a "constant" medium of hay infusion unfavorable because it lacks some elements which are essential for the continued existence of this protozoon? To test this point beef extract was chosen because it was believed that it contains all of the essential elements. After a series of preliminary tests, a .025 per cent. solution of Liebig's extract of beef was decided upon for the experiments.

Briefly, it may be stated that the solution of beef extract employed has proved (during the seven and one half months of the experiment) to be practically as favorable a medium for the reproduction of the pedigree culture of *Paramœcium aurelia* as the "varied" culture medium; and therefore the conclusion seems justified that this culture can, in all probability, be continued indefinitely on this medium, and that it is the "composition" of the medium rather than "changes" in the medium which is conducive to the unlimited development of this culture without conjugation or artificial stimulation. It is also believed that a "constant" medium of beef extract, in this or similar solutions, will prove to be a favorable culture medium in which to breed pedigree cultures of paramæcia for certain physiological studies,

since it is clear from many investigations that the reactions of paramæcia to various stimuli are greatly modified by the past and present environment of the organisms.

74 (599)

Conjugation of closely related individuals of Stylonychia.

By **GEORGE ALFRED BAITSELL.**

[*Sheffield Biological Laboratory of Yale University.*]

A "wild" *Stylonychia pustulata* was isolated from a laboratory aquarium on October 1, 1910, and placed on a "constant" beef extract medium. This culture, which consisted of four lines, was kept on depression slides and the animals were isolated daily. The medium proved to be a favorable one for this animal and in a period of about four months the culture reached the 350th generation. At that time (February 5, 1911), when for ten days they had been dividing at an average rate of over three divisions per day (which was the highest rate of division that they had attained) a considerable number of conjugations between closely related cells occurred in the "stock" of the culture left over from the daily isolations. For a period of about three weeks this phenomenon was quite general in the stock and apparently would occur whenever a sufficient number of animals were present on a slide. To study the effects of conjugation, 132 conjugating pairs were isolated. These were kept in exactly the same kind of medium as that in which the conjugation had occurred so that the character of their environment was not changed by the isolation. From over 90 per cent. of the isolated conjugating pairs, ex-conjugants were obtained (after a union of the usual duration) which were perfectly normal in general appearance and behavior. However, none of these ex-conjugants divided and none lived 48 hours after separating. Animals obtained from "split" conjugating pairs also died without dividing. It was impossible to prolong the life of the ex-conjugants by any of the methods tried. Also from the time that the epidemic of conjugation made its appearance there was a continuous and rapid fall in the division rate of the main lines of the culture when averaged for ten-day

periods, and by the 25th day following, the entire culture had died out. In another culture, which was started from this one at the 150th generation and kept on a hay infusion medium, conjugation did not occur and this culture is still alive.

75 (600)

The cultivation of tissue in plasma from alien species.

By **ROBERT A. LAMBERT** and **FREDERIC M. HANES.**

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University.*]

The present series of experiments have been concerned with the attempt at cultivating in vitro rat sarcoma, rat spleen and mouse carcinoma, in plasma obtained from animals of other species, and at analyzing the factors contributing to the phenomena observed.

In a former note we recorded the fact that mouse and rat plasma could be interchanged as culture media for the tumors of these species, but that growth seemed to be more vigorous when homologous plasma was used. Guinea pig, rabbit, dog, goat, human and pigeon plasmas have been employed in the studies herewith reported.

For determining the viability of tissue under the conditions of the experiment we have made transfers of the pieces to homologous plasma—a rapid and satisfactory test. Animal inoculations have also been made in the case of tumor tissue, but aside from the delay in noting the results the procedure has other objections.

As a culture medium for rat sarcoma guinea pig plasma is only slightly less suitable than rat plasma, the difference consisting chiefly in the extent of the out-wandering of cells. The cells may remain viable in a single drop of plasma for twelve days or more; we have had pieces which showed marked activity after a month's sojourn in several drops of the alien medium. Mouse carcinoma seems to grow almost as well in guinea pig plasma as in rat plasma. Mitoses have been observed after eight days, and cultures nine days old produced tumors when inoculated into mice.

Rabbit plasma is distinctly less suitable for the growth of mouse

and rat tumors. Liquefaction about the pieces of tissue is often quite marked. With sarcoma the growth, though relatively slow, may continue for ten to twelve days.

In dog plasma pieces of sarcoma, after one or two days, present a fairly diffuse radial outgrowth of clear spindle cells, which after this time undergo rapid disintegration. About the pieces there is noted a narrow clear zone (liquefaction). With mouse carcinoma liquefaction is more marked and there is little or no outwandering of cells.

We have not observed any of the phenomena of growth when using goat plasma. After a few hours there is seen surrounding the pieces of tissue a wide granular zone. Liquefaction of fibrin does not occur.

In human plasma the most striking and constant phenomenon is the progressive liquefaction of the fibrin, which is practically complete after six or seven days. In spite of the loss of framework there takes place, however, an active migration of cells, which wander out along the cover glass, reaching often the edge of the medium. As a rule the cells move out separately, but we have observed in several preparations of rat spleen radial out-growths simulating the appearance seen in homologous plasma where the fibrin network is preserved. In the single cells attached to the cover glass the most interesting changes have been noted, especially in the cultivation of rat spleen. After four or five days cells of extraordinary size begin to appear, reaching a maximum size in two or three days. Such a "giant cell" examined in the fresh state shows about its centre a clear nuclear area, with or without knob-like prominences, surrounded by a highly granular zone, which in turn merges into a clear filmy indefinite protoplasm with processes. In their entire extent these cells vary from 100 to 700 mikra in diameter. When stained the knob-like prominences are seen to be nuclei with distinct nuclear membranes and prominent nucleoli. These nuclei are often seen in close apposition arranged about a light hematoxylin staining, reticular or vacuolated area. In the fresh this portion of the cell was interpreted as nucleus. Many large cells varying considerably from these were also seen. Quite large multinucleated cells have been observed in the growth of sarcoma and carcinoma in rat plasma but none that simulated

those just described. Pieces of rat spleen or tumor when transferred to rat plasma after five or six days in human plasma begin to grow very actively. The cells, however, often present extremely ragged outlines. Giant cell formation may be noted after the transfer.

The growth of rat sarcoma in pigeon plasma is especially beautiful and appears to be quite characteristic. During the first four or five days there is a progressive radial extension of large clear spindle cells of strikingly uniform size and morphology. The strings of cells are connected by long slender processes. Fine granular fat accumulations in the protoplasm appear early and increase in size and number with the age of the specimen, just as occurs in rat and guinea pig plasmas. As a rule no further growth takes place after the fifth day, and the cells at this time begin to show signs of disintegration. Intact well stained nuclei, however, may sometimes be seen in preparations eight or nine days old. Transfer of the pieces of tissue in four-day specimens to fresh pigeon plasma does not result in a prolongation of the period of activity.

In summarizing the above findings we notice that goat plasma is the only medium used in which there was no growth; in dog plasma growth was of short duration. Arranged in the order of suitability we have guinea pig, rabbit, pigeon, human, dog and goat. In a study of cytolysins for rat and mouse tissues we found that hemolysins were present in human, dog and goat sera. The sera tested were taken from animals from which plasma had been obtained for tissue cultivation. We have found also that in plasma from guinea pigs immunized against rat corpuscles, growth of rat sarcoma does not occur, or is of an abortive character, and that there is a similar inhibition of growth in plasma from guinea pigs previously treated with large doses of rat sarcoma.

76 (601)

The behavior of fat-soluble dyes in the organism.

By **LAFAYETTE B. MENDEL** and **AMY L. DANIELS.**

[*From the Laboratory of Physiological Chemistry, Sheffield Scientific School, Yale University, New Haven, Conn.*]

It is well known that the fat-soluble dye, Sudan III., is readily deposited in the adipose tissue of animals. An attempt was made by the authors to study the movements of the dye under conditions where fat transport takes place (*e. g.*, in starvation, phlorhizin- and phosphorus poisoning). The dye readily migrates into the blood with the fat under these conditions, but is rarely found in the liver tissue into which large quantities of fat enter (fatty infiltration). This is explained by the observation that the Sudan III. is abundantly excreted with the bile into the intestine from which it may be reabsorbed. Sudan III., which is insoluble in water, is not excreted through the kidneys except where alimentary lipuria is induced (in rabbits and rats). The elimination from the liver is not accomplished through the solvent medium of fat excreted in the bile (lipocholia); but the dye is soluble in bile as well as in solution of the isolated bile salts. *We have thus established a path of elimination for fat-soluble (or bile-soluble) substances through the biliary secretion.* An investigation of a considerable number of water-insoluble, fat-soluble compounds—mostly non-toxic aniline dyes and food colors—showed comparable conditions justifying the above general conclusion. It has further been established that these water-insoluble compounds do not experience absorption from the intestine in the absence of bile. Dissolved in fat-emulsion and introduced into the organism by alimentary, subcutaneous, or intravenous paths, these dyes are always eliminated with the bile into the intestine. When there is a paucity of fat in the diet the fat-soluble dyes may be absorbed through the agency of reabsorbed bile, but they are speedily eliminated again by the liver channels; with an abundance of fat to act as carrier, they travel with it through the lymphatics into the circulation. The distribution of fat-soluble dyes within the organism depends on the presence of fat and its migrations.

Thus they may be carried to or from adipose tissues, be deposited in the egg-yolk, or be secreted in company with fat in the milk of animals; they apparently do not traverse the placenta. The dyes have not been detected in the lipoids of the nervous tissue. We have failed to note any inability on the part of animals to utilize fats in which Sudan III. has been deposited.

77 (602)

Experimental studies on creatine and creatinine.

By **W. C. ROSE.**

[*From the Laboratory of Physiological Chemistry, Sheffield Scientific School, Yale University, New Haven, Conn.*]

The excretion of creatine induced by starvation in rabbits, is inhibited partially or completely by feeding a diet of carbohydrates alone. The creatine elimination is not reduced by feeding a diet of fat alone or by a diet of fat and protein.

Experimental interference with carbohydrate metabolism leads to the elimination of creatine. After phlorhizin diabetes, which depletes the store of carbohydrates, and during phosphorus poisoning, which disturbs the glycogenic functions, the output of creatine in dogs is decidedly increased.

An increase in the output of creatine plus creatinine (total creatinine) is always accompanied by an increase in total nitrogen elimination. This parallelism in inanition and with nitrogen-free diets, is ascribed to a common source,—namely, true tissue or endogenous metabolism. The metabolism of exogenous or reserve proteins is not accompanied by the production of creatine or creatinine.

Coincident with the increased elimination of total creatinine during fasting, a significant increase in the creatine content of muscle occurs in rabbits and hens. This indicates an increased production of creatine during the accelerated catabolic processes.

Creatine is a normal constituent of the urine of the young until the age of puberty. Possibly this is due to insufficient glycogenic functions. Though no direct evidence for such an assumption has been obtained, still the ease with which children develop

glycosuria and acidosis, and the rapidity with which they succumb to diabetes, renders such an explanation probable. It is conceivable also that during the period of growth the demand for carbohydrates for the histogenetic processes may be so great that the cells are left in partial carbohydrate hunger, and are unable to perform the "endo-catabolic" activities as perfectly as in later life.

Without question the metabolism of creatine is intimately associated with carbohydrate metabolism.

78 (603)

The rate of tumor growth in underfed hosts.

By **PEYTON ROUS.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*]

Workers with transmissible neoplasms have had frequent occasion to observe that sick or emaciated animals are relatively resistant as hosts for implanted tumor. It does not develop in them with the same readiness as in healthy individuals. A kindred phenomenon has been noted by Moreschi[1] in studying the relation of nutrition to tumor growth. He found that in mice losing weight on a low diet an engrafted sarcoma survived with less frequency and grew more slowly than in the well-fed controls. Indeed these controls died of their tumor sooner than did the fasting animals.

This being so might it not be possible to delay by food-restriction the course of inoperable tumors? And might not the development of metastasis after excision of a primary growth be hindered by the same means? In an attempt to answer these questions the author has performed a series of experiments with the Flexner-Jobling adeno-carcinoma of the rat. This neoplasm in its invasive spread and tendency to metastasize has a striking likeness to some of the cancers of human beings.

A bread compounded of oatmeal, rye-flour, corn-meal, milk and sugar, was baked in large quantity, dried, ground, and, with sufficient milk to moisten it, was used as the sole food of the

[1] C. Moreschi, *Zeitschr. f. Immunitätsforsch.*, 1909, VI., 651.

experiment animals. Preliminary observations were made on several series of healthy rats to determine how little of the bread would sustain life while permitting of a gradual emaciation, and then 110 young, growing animals, carrying the Flexner-Jobling tumor, were submitted, half to food restriction, while the other half were fed full and used as controls. The food was carefully measured out, the exact size of the tumors charted each week, and the rats weighed twice a week. Moreschi observed the effect of food restriction from the time of the tumor's implantation or shortly thereafter until the host's death. For our purpose animals were mainly used in which the tumor had attained a large size before the restricted diet was begun. Contrary to expectation it was found that these large tumors continued to grow with the same rapidity in hosts emaciating on a restricted diet as in the controls, many of which were still gaining weight. With yet larger tumors and hosts already cachectic food-restriction had also no effect on the primary growth, and none on the frequency of metastasis formation.

Moreschi's findings were confirmed so far as regards the influence of poor nourishment of the host to hinder the development of tumor grafts. And when food restriction was begun a few days (four) after the introduction of the grafts it was noted that the tumors developed a little more slowly than in the controls. It is easy to understand how, given such a retarding influence on the tumors early in their career, the experiment animals might outlive the controls, as Moreschi found to be the case. The sarcoma he used was probably more susceptible than our carcinoma to changes in the host's nutrition.

The fact that large tumors take their course irrespective of the condition of nourishment of the host does not at present admit of direct explanation any more than does the phenomenon of regeneration in starving animals. It is to be correlated with the clinical observation on malignant disease that the tumor often grows with great rapidity despite a marked progressive emaciation of the patient. Our experiments have not yet answered the important question as to whether in animals from which the primary tumor has been removed the metastases will behave like tumor grafts in that their growth is slower in ill-nourished hosts; or

whether, as is the case with large primary tumors at this time, the growth will go on independently of the host's condition of nourisment.

79 (604)

Preliminary report upon the transmission of haemolysins from mother to offspring.

By **L. W. FAMULENER.**

[*From the Research Laboratory, Department of Health, New York City.*]

In view of the fact that little work has been done upon the transmission of hæmolysins from mother to offspring, and that there is a lack of agreement between the reported results of the workers in this field, the subject was taken up for further investigation. The question is of practical importance, since a parallel relationship exists between hæmolysins and bacteriolytic bodies: the latter group of substances play a more or less important rôle in immunity against certain infections.

Goats were selected as the most suitable experimental animals for these studies. In each case the animal was actively immunized by repeated injections of washed sheep-corpuscles given subcutaneously. Serum and milk samples were collected, throughout each experiment, and stored in an ice-box. All were tested at the same time, under uniform conditions, after the given experiment was closed.

One series of animals were immunized immediately following the birth of their young. In all, excepting one case, the milk contained no demonstrable hæmolysins. As evident, the sucklings from the mothers which supplied the negative milk, gave negative sera. The suckling which received the mother's milk containing hæmolysins, showed no specific hæmolysins at any time in its serum. The hæmolysins did not appear (by test) in the milk in this case until about one week after birth of the young.

A second series of animals were immunized at different periods during the course of gestation. Before birth of the young the animal's nipples were sealed to prevent the young getting any

milk before blood samples were taken. When twins came, one was placed, at once, upon cow's milk for control. At the time of birth none of the young showed specific hæmolysins in the blood serum. But those getting the colostrum and first milk rapidly acquired, and retained, the specific antibodies. The colostrum in those cases was very rich in hæmolysins, but the antibodies disappeared from the milk output after a few days, in so far as we were able to ascertain.

80 (605)

The effects of intraperitoneal injections of adrenalin on the partition of nitrogen in the urine of dogs.

By **JACOB ROSENBLOOM** and **WILLIAM WEINBERGER.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

Underhill and Closson have shown that the *subcutaneous* injection of adrenalin chlorid solutions into dogs is not attended by any significant change in the proportion of the urea, ammonia and creatinin nitrogen of the urine.

In our work we used two specimens of the colorless adrenalin chloride, 1:1000, of Parke, Davis and Co. They were purchased in the open market. Each was tested for its pressor action at the conclusion of the corresponding injection experiments and was then found to be practically as active as ever.

The metabolism experiments were carried out by the methods in use in this laboratory.

Intraperitoneal injection of adrenalin chloride solutions was without effect on the proportions of nitrogen in the forms of urea, ammonia, creatin and creatinin, purins and allantoin.

In one adrenalin injection period of eighteen days, a total of 62 c.c. of a 1:10,000 adrenalin solution was given intraperitoneally and in another injection period of six days a total of 29 c.c. of a 1:1000 adrenalin chloride solution was administered.

The following table shows the different percentages of nitrogen for the several experimental periods:

PERCENTAGE OF TOTAL NITROGEN.

Periods.	Urea N, per cent.	Ammonia N, per cent.	Creatin and Creatinin N, per cent.	Purin N, per cent.	Undetermined N, per cent.
I. (Fore period)	88.5	3.64	2.51	0.16	5.19
II. (First injection period)	85.7	3.75	2.50	0.20	7.86
III. (First post injection period)	86.5	3.61	3.33	0.24	6.32
IV. (Fore period)	86.7	3.65	3.20	0.19	6.26
V. (Second injection period)	87.0	3.92	3.73	0.16	5.19
VI. (Second post injection period)	84.2	4.29	3.62	0.17	7.72

81 (606)

Experiments on the diffusibility of cholesterol-esters and of lecithan compounds.[1]

By **ERNST BOAS** and **JACOB ROSENBLOOM.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

I. CHOLESTEROL ESTERS.

It has been shown in this laboratory that ether solutions of various biological substances pass through rubber membranes into ether.

We have found that cholesterol-benzoate, cholesterol-stearate, cholesterol-oleate and cholesterol-palmitate dissolved in ether will readily diffuse through rubber into ether.

Cholesterol-stearate with a molecular weight of 652.61 diffuses, whereas the various lecithans, with molecular weights considered to be 770 to 785, do not. If we assume that the diffusion of a substance depends on the size of its molecules, the above facts strengthen Hiestand's conclusion that the molecular weight of egg-yolk lecithin is 1446, which figure he obtained by a molecular weight determination.

[1] This study is one of a projected series on *lipins*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

II. LECITHAN COMPOUNDS.

Koch[1] has lately described the preparation of various compounds with lecithans, but it is uncertain whether these compounds are colloidal mixtures, mechanical mixtures or true chemical compounds. It seemed of interest to study the behavior of these substances in ether solution, when subjected to dialysis in rubber bags suspended in ether.

The preparations used in these experiments were made according to the method described by Koch. For the dialysis tests the solutions of the lecithan compounds were evaporated to dryness at 38° and the residues ground up with ether. The extracts were filtered and the filtrates placed inside of rubber bags and dialyzed against ether for thirty-seven days. The dialysates were tested every week to see if the substance combined with the lecithan diffused.

Compounds of lecithin with glucose, lactic acid, strychnin, digitonin, salicin, urea, creatin, creatinin and caffein were prepared. It was found that the glucose and lactic acid dialyzed completely, the strychnin, digitonin and salicin dialyzed partially, while urea, creatin, creatinin and caffein did not dialyze at all.

82 (607)

The relative importance of stroma and parenchyma in the growth of certain organs in culture media.

By **MOYER S. FLEISHER** and **LEO LOEB.**

[*From the Departments of Pathology of the Barnard Free Skin and Cancer Hospital and the City Hospital of St. Louis, Mo.*][2]

In experiments carried out about fifteen years ago, one of us observed that during the regeneration of skin, the epithelial cells are able to penetrate into and to grow in coagula of blood and of blood plasma.[3] This suggested to him that it might be

[1] Koch, *Journ. Pharm. and Exp. Ther.*, 1911, ii, p. 239.

[2] We wish to express our thanks to Dr. D. L. Harris, director of the Pathological Laboratory at the City Hospital, who put the facilities of his laboratory at our disposal at a time when our laboratory had not been finished; and also to Dr. M. G. Seelig, who very kindly assisted us in a number of our experiments.

[3] *Archiv f. Entwicklungsmechanik*, Bd. VI., 1898; *Johns Hopkins Hospital Bulletin*, January, 1898; *American Journal of Anatomy*, Vol. III., 1904.

possible to make various tissues grow in culture media outside of the body, in the thermostat, as well as inside the body, in the latter case the body acting as a thermostat. Inasmuch as he noted that the epithelial and also connective tissue cells grew preferably in contact with solid structures as fibres of fibrin, and into solid gelatinous material rather than into fluids he attributed stereotropic sensitiveness to various tissue cells, and he consequently employed more or less solid culture media as agar and coagulated blood serum for his various experiments. At first he carried out experiments in vitro as well as experiments in which the animal body acted as an incubator. Lack of the necessary facilities made it very soon necessary for him to limit himself to the latter kinds of experiments.[1]

To our knowledge in these our earlier experiments for the first time the attempt was recorded in the literature to grow tissues of higher animals under artificial conditions in environments that differ from those found in the body under natural conditions, to separate experimentally growing epithelial from connective tissue cells, and furthermore to study the influence of the addition of certain chemicals upon the growth of tissues.[2] Thus among other facts it was found that epithelial cells can grow in these gelatinous culture media even after addition of certain salts; that this growth can take place independently of connective tissue cells, that the epithelial cells may divide mitotically in the culture media, that they invade the coagulum through ameboid movements, that they have the power of phagocytosis, taking up into their body small particles of the culture medium;[3] that the growth ceases after this period of activity, and we explained on the basis of our observations certain phenomena of cancer growth as due to active ameboid ingrowth of cancer cells into the deeper tissues.[4] Later Harrison[5] showed in most interesting experiments that it is possible to grow embryonic nervous tissue of the frog in a mixture of fibrin and serum of the frog lymph, and recently Burrows

[1] Chicago, 1897; *Archiv f. Entwicklungsmechanik*, Bd. XIII., 1902. *The Journal of Medical Research*, Vol. VIII., 1902; *Journal Am. Med. Association*, 1901.

[2] *Zeitschrift f. Krebsforschung*, Bd. V., 1907, pp. 14 and 15.

[3] *Archiv f. Entwicklungsmechanik*, Bd. XIII., 1902.

[4] *Johns Hopkins Hospital Bulletin*, January, 1898.

[5] PROCEEDINGS SOC. EXP. BIOL. AND MED., Vol. III., 1907; *Journal Experimental Zool.*, Vol. IX., 1910.

extended in Harrison's laboratory the use of fibrin and blood serum as a culture medium to the growth of various mammalian tissues. Other investigators especially Carrel and his collaborators have within the last nine months taken up these studies and grown tissues in fibrin as well as in agar. We also resumed recently our former experiments and here we wish to report briefly a few of our results as far as they concern the relative importance of stroma and parenchyma in the tissue growth in gelatinous culture media:

A. After transplantation in vitro as well as in vivo, the central parts of stroma and parenchyma become necrotic, only the peripheral parts remaining alive. The extent of this central necrosis varies in different organs; it is, for instance, greater in the case of the kidney than in the case of the testicle.

B. In regard to the relative importance of stroma and parenchyma in the growth of certain organs in coagula we notice certain differences in various organs.

1. In the ovary of the guinea pig and rabbit either the whole piece becomes necrotic or in other cases the connective tissue and some follicles of the cortex remain entirely or partly alive. The epithelial covering of the ovary remained alive and even proliferated in a few cases. Mitoses in stroma or parenchyma cells or distinct ingrowth of connective tissue or epithelium into the coagulum were not observed. The greater part of the tissue became necrotic.

2. In the case of the testicle of the rabbit the peripheral alveoli usually remain alive without however any new formation of spermatozoa taking place. An irregular development of some epithelial cells into multinucleated giant cells may occasionally be observed. An outgrowth of the parenchyma into the coagulum does not occur, while the connective tissue can grow very actively into the coagulum.

3. In the case of the kidney an outgrowth of connective tissue into the coagulum was a very frequent occurrence especially in cases in which in the periphery of the transplanted piece a part of the connective tissue capsule had remained adherent to the parenchyma. The tubular epithelium in the periphery of the transplanted piece usually remains alive. Growth in the parenchyma takes principally place within the area of the transplanted

tissue, the regenerating cells of the tubules pushing the older cells into the lumen of the tubule and such desquamated necrotic cells glue together and form casts. Tubular epithelium can also grow between the transplanted piece of kindey and the coagulum and sometimes it penetrates into the coagulum forming occasionally canals in the latter. Mitoses are seen in the proliferating cells of parenchyma and stroma and such cells dividing mitotically may be seen lying directly in the coagulum. This description holds good for the ordinary manner of experimentation. Under certain conditions which we expect soon to describe in more detail it seems possible to increase very markedly the proliferating and infiltrating activity of the tubules.

4. In the case of the mammary carcinoma of the mouse the growth of the parencyhma is very much more prominent than in the case of the normal organs examined, a fact that is in accordance with the rapid growth of carcinoma cells in contact with animal cells in the body.

5. While various authors state that after the ordinary transplantation of carcinoma of the mouse into the subcutaneous tissue the stroma perishes and only the parenchyma remains alive, after transplantation into the culture media the stroma of mouse carcinoma remains alive and shows even certain growth phenomena. This observation should suggest a renewed investigation of the fate of the stroma after transplantation of carcinoma into the subcutaneous tissue of an animal.

6. Both parenchyma and stroma of the carcinoma of the mouse grow approximately equally well in coagulated blood plasma of the rat and of the rabbit.

7. The parenchyma cells of carcinoma penetrate very much more frequently into the coagulum than the parenchyma cells of other organs investigated so far. Mitoses are frequently seen in the carcinomatous cells and they may even be found in cells lying in the coagulum.

8. In regard to the skin we observed in our early experiments published elsewhwere the epithelial cells to penetrate in relatively large masses into the coagulum and we furthermore described at that time mitoses in these infiltrating epithelial cells.

C. We also noticed that the epithelial cells of the skin may

become phagocytic and take up small particles of agar and coagulated blood serum. Similar observations we made recently in the case of growing tubular cells of the kidney and of carcinomatous cells[1] and probably also in the case of ingrowing stroma cells.

D. While the parenchymatous cells penetrate onto the coagulum in a more or less definite arrangement which to some extent corresponds to the normal arrangement of the parenchyma cell, the form of cell columns or of tubules being retained respectively, the connective tissue cells on the other hand grow as single cells often sending out long drawn out stellate processes in the coagulum, the various connective tissue cells being loosely connected by such processes. This arrangement enables the stroma cells to penetrate into the coagulum with relatively greater ease than is possible in the case of parenchyma cells.

E. Stroma as well as parenchyma cells (including carcinoma cells) show a definite direction in which they grow into the coagulum, both having the tendency to proceed along the fibers which form in the coagulum and to follow, if at all, for a short distance only a course vertical to the direction of the fibers in the coagulum. The connective tissue cells seem however in consequence of their isolated and independent mode of growth and of moving occasionally to be able to penetrate into the coagulum in other directions more easily than parenchyma cells, but they also usually follow the road of least resistance.

F. We recognize therefore variations in the relative importance of stroma and parenchyma in the case of the growth of different organs. In a provisional way we may assume that the parenchyma of those organs or tissues that normally show an additive (expansive) growth (active outgrowth) like carcinoma and stratified epithelium of the skin show likewise an infiltrative growth in coagula (in gelatinous culture media generally) and that those organs that normally or during regeneration do not show additive (active expansive out) growth, but rather compensatory hypertrophy, do correspondingly not show a strong tendency to infiltrative growth, when growing in culture media (testicle, ovaries). In the latter class however the stroma may or may not show infiltrative

[1] The same observation has been recently made in the case of tumor cells by R. A. Lambert and F. M. Hanes, Journal Exp. Med., Vol. XIII, 1911, p. 495.

growth. Certain other organs like kidney apparently hold an intermediate position. Here both parenchyma and stroma may grow into the culture medium; the growth of the parenchyma is however under ordinary conditions relatively slight.

There are however in all probability other factors of importance besides the one just mentioned. Thus we observed so far in our experiments a very much more active growth of the connective tissue of the testicle than of the ovary. Perhaps the difference in texture of the organs is in this case one of the determining factors, the looser texture of the testicle being more favorable to the outgrowth of the stroma than the dense structure of the ovary.

We furthermore found differences and similarities in the manner of growth of stroma and parenchyma in the coagulum.

83 (608)

A possible significance of the Cammidge reaction.

By **L. B. STOOKEY.**

[*From the Physiological Laboratory, Medical School, University of Southern California.*]

Smolenski[1] attributes the Cammidge reaction to saccharose. This led us to think of some intestinal lesion as a possible source of the Cammidge reaction. Two possibilities seem to be evident (1) absorption of saccharose as such, (2) reversible action of intestinal saccharase.

To test this view the Cammidge test was made on urines in cases of "chronic intestinal disturbance." Twelve cases, only one of which showed a clinical suspicion of a pancreatitis, were studied. Five gave a positive Cammidge reaction. The case showing probably the most pronounced reaction failed to give the Cammidge test after 48 hours' starvation. During the twelve hours following the starvation period a liberal quantity of milk sweetened with levulose was given. This did not lead to a positive Cammidge.

From the experiments made thus far it seems probable that in cases showing a positive Cammidge there may be some relationship between the amount of cane sugar ingested and the intensity of the Cammidge reaction.

[1] *Zeitschrift für physiol. chem.*, 51, p. 127.

84 (609)

The relation of the fixation reaction to specific precipitation.

By **FREDERICK P. GAY.**

[*University of California.*]

This work represents a continuation of studies on the mechanism of the fixation reaction between a serum and its antiserum begun by the author in 1905. It was shown that this fixation which had been attributed by Gengou to the presence of anti-albuminous sensitizers was apparently produced by the precipitate formed in the serum-anti-serum mixture. A prolific literature has since sprung up engaged principally in establishing the presence or absence of parallelism between fixation and precipitation. The results in either direction are far from conclusive.

In the present work an attempt has been made to settle the question by studying more attentively an instance in which both precipitation and fixation are known to occur, and where one may reasonably be associated with the other. It is found that in addition to the voluminous precipitate which is known to be formed by mixture of an excess of the antiserum with the antigenic serum, there is another precipitate produced with certain individual antisera in the presence of a large excess of antigen. This latter, and we believe newly recognized, zone of precipitation lies above the zone of inhibition produced, as is well known, by the ordinary excess of antigen. The upper zone precipitate differs in its granular appearance and slowness of formation from the lower zone precipitate.

No fixation of alexin occurs as a rule in mixtures representing the inhibition zone and never in presence of the upper-zone precipitate which may, however, equal or exceed in volume a precipitate of the lower zone which produces complete fixation. This fixation in the lower zone precipitate mixture is produced almost invariably by the washed precipitate and not by the supernatant fluid. The latter fluid may, however, in some cases give partial fixation.

It is well recognized that the addition of a small amount of antigen to the formed precipitate will dissolve it. This applies

to the lower zone precipitate only. A solution of the precipitate by this method gives a perfectly clear solution which fixes as well as the formed precipitate. In similar manner solution of the precipitate by traces of acid or of alkali gives a fixing fluid equal in potency to the undissolved precipitate; the latter method is, for technical reasons, less conclusive than the former method of solution.

The conclusion to be drawn from this work is that alexin fixation by a mixture of serum and antiserum is produced by an antigen-antibody complex distinct from precipitinogen-precipitin but usually brought down by the precipitate in its formation in such a way as to give the appearance that the fixation is produced by the precipitate itself. It would seem then that Gengou's original supposition, without direct proof, of anti-albuminous sensitizers more correctly explains this type of the fixation reaction. The best expression of the conditions as they seem to exist would be found, however, in Nicolle's hypothesis who concludes that antisera contain two classes of antibodies, "coagulins" and "lysins," the former, in this particular instance, producing precipitation, and the latter, the fixation reaction.

RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

VOLUME VIII.

Donaldson, H. H.

575. On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog.

Eisenbrey, A. B. [with **R. M. Pearce.**]

581. The depressor action of dog's pancreas and pancreatic juice.

Famulener, L. W.

604. Preliminary report on the transmission of hæmolysins from mother to offspring.

Fedde, Nathanael.

544. A blood pressure apparatus with pith-ball attachment indicating diastolic pressure.

Fisher, H. L.

543. See Foster, N. B.

Fitzpatrick, C. B.

540, 549. See Atkinson, J. P.

Fleisher, M. S. [with **Leo Loeb.**]

607. The relative importance of stroma and parenchyma in the growth of certain organs in culture media.

Flournoy, Thomas.

591. See Steinhardt, Edna.

Foster, N. B.

542. Creatin metabolism during convalescence from typhoid.

543. [with **H. L. Fisher.**] Creatin and creatinin metabolism in dogs with Eck fistula.

Frost, W. H.

556. See Anderson, J. F.

Gay, Frederick P.

609. The relation of the fixation reaction to specific precipitation.

Gettler, A. O.

596. See Sherman, H. C.

Gies, W. J.

566. See Rosenbloom, J.

567, 589. See Goodridge, F. G.

568. A demonstration of the diffusion of pigments from fat through rubber into fat.

588. See Sidbury, J. B.

536. Simultaneous graphic registration of gastric and duodenal peristalsis in rabbits.

547. The influence of calcium and of sodium in *m*/10 solutions upon the conductivity in nerve trunks.

584. On the convulsant action of acid fuchsin (Abel and Barbour) in cardiectomized frogs.

Karsner, H. T. [with **J. B. Nutt.**]

590. The relation of the toxic dose of horse serum to the protective dose of atropine.

Kendall, E. C.

597. The determination of small amounts of iodine in organic combination—A modification of Hunter's method.

King, Helen D.

577. Experiments to modify the sex ratio in the toad.

Kleiner, I. S.

564. The elimination of glucose into the gastro-intestinal canal.

585. [with **S. J. Meltzer.**] On the presence of dextrose in the exudate of pulmonary edema.

Lambert, R. A.

558. [with **F. M. Hanes.**] A comparison of the growth of sarcoma and carcinoma cultivated in vitro.

594. See Hanes, F. M.

600. [with **F. M. Hanes.**] The cultivation of tissue in plasma from alien species.

Levin, I. [with **M. J. Sittenfield.**]

595. The formation of metastases after an intravascular injection of tumor emulsions.

Lillie, Ralph S.

578. Evidence that the primary change on stimulation is an increase in the permeability of the limiting membrane.

Loeb, Jacques.

531. The influence of the concentration of hydroxyl ions on the antagonistic effect of salts with bivalent metals.

Loeb, Leo.

539. See White, Ellen P. Corson.

607. See Fleisher, M. S.

Lusk, Graham.

560. See Williams, H B.

second inoculation with tumor material of experimentally increased virulence.

White, George F.

532, 533. See Van Slyke, D. D.

Williams, Anna W.

557. Pure cultures of parasitic amebas on brain-streaked agar.

Williams, H. B.

559. A respiration calorimeter of the Atwater-Rosa-Benedict type, designed for use with dogs and children; with demonstration.

560. [with **J. A. Riche** and **Graham Lusk.**] The chemical and energy transformations in the dog after the ingestion of different quantities of meat.

Wolf, C. G. L. [with **Ernst Sachs.**]

546. Metabolism after hypophysectomy.

Woodruff, L. L.

586. The effect of culture medium contaminated with the excretion products of Paramæcium on its rate of production.

598. [with **G. A. Baitsell.**] Beef extract as a "constant" culture medium for *Paramœcium aurelia.*

EXECUTIVE PROCEEDINGS.

Fortieth meeting.

College of Physicians and Surgeons, Columbia University, October 19, 1910. President Morgan in the chair.

Members present: Atkinson, Auer, Banzhaf, Beebe, Benedict, Berg, Calkins, Field, Goldfarb, Hatcher, Hunter, Jackson, Joseph, Lee, Levene, Levin, Loeb, J., Mandel, J. A., McClendon, Meltzer, Meyer, Morgan, Murlin, Norris, Opitz, Ringer, van Slyke, Stewart, H. A., Stockard, Storey, Swift, Terry, Wadsworth, Wallace, Weil.

Members elected: John Howland, R. A. Lambert, Ernst Sachs, H. B. Williams.

Forty first meeting.

The Rockefeller Institute for Medical Research, December 21, 1910. President Morgan in the chair.

Members present: Auer, Dochez Emerson, Janeway, Joseph, Gies, Hawk, Lamar, Lusk, Meltzer, Sachs, Steinhardt, Stockard, Swift, van Slyke, Wallace, Wolf.

Members elected: F. J. Birchard, Charles B. Fitzpatrick, Charles E. A. Winslow.

Forty second meeting.

Cornell University Medical College, February 15, 1910. President Morgan in the chair.

Members present: I. Adler, H. M. Adler, Auer, Banzhaf, Beebe, S. R. Benedict, Calkins, Dochez, Dunham, Ewing, Famulener, Field, Gies, Joseph, Lambert, Lusk, J. A. Mandel, A. R. Mandel, Meltzer, Morgan, Ringer, Sachs, Terry, van Slyke, Wallace, Weil, H. B. Williams, A. W. Williams.

Members elected: W. F. Longcope, F. M. McCrudden, H. O. Mosenthal, Jacob Rosenbloom.

Officers elected: President, Thomas H. Morgan; Vice-President, P. A. Levene; Secretary, George B. Wallace; Treasurer, Graham Lusk.

Forty third meeting.

University of Pennsylvania, Laboratory for Hygiene, April 19, 1911. President Morgan in the chair.

Members present: Abbott, Auer, Cook, Donaldson, Eisenbrey, Goldfarb, Hatai, Huber, Joseph Levin, Lillie, Longcope, Lusk, Mandel, Meltzer, Morgan, Pearce, Reichert, Ringer, Taylor, Terry, Wallace, Wolf.

Members elected: Alfred E. Cohen, T. H. Montgomery.

Forty fourth meeting.

University and Bellevue Hospital Medical College. May 17, 1911. President Morgan in the Chair.

Members present: Auer, Benedict, Cole, Famulener, Field, Foster, Gies, Jackson, Joseph, Lambert, Levin, MacCallum, Mandel, Meltzer, Mendel, Morgan, Pappenheimer, Ringer, Rosenbloom, Rous, Sherman, Storey, Steinhardt, Swift, Terry, Wallace.

Members elected: F. W. Bancroft, D. H. Bergey, W. H. Frost, J. S. Githens, H. T. Karsner, I. S. Kleiner, W. H. Manwaring, E. B. Meigs, R. Ottenberg.

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KARSNER, H. T.....University of Pennsylvania.
KAST, LUDWIG.....New York Postgraduate Medical School.
KASTLE, JOSEPH H.....U. S. Public Health and Marine-Hospital Service, Hygienic Laboratory, Washington, D. C.
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LEVENE, P. A.....Rockefeller Institute for Medical Research.
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LOEB, LEO.....Barnard Cancer Hospital, St. Louis.
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MACALLUM, A. B.....University of Toronto.
MACCALLUM, W. G.....Columbia University.
MACDOUGAL, D. T.....Carnegie Institution, Washington, D. C.
MACLEOD, J. J. R.....Western Reserve University, Cleveland.
MACNEAL, WARD J.....University of Illinois.
MCCLENDON, J. F.....Cornell University Medical College.
MCCRUDDEN, F. M.....Rockefeller Institute for Medical Research.
MALL, FRANKLIN P.....Johns Hopkins University.
MANDEL, ARTHUR R.....New York University.
MANDEL, JOHN A.....New York University.
MANWARING, W. H.....Rockefeller Institute for Medical Research.
MATHEWS, ALBERT P.....University of Chicago.
MAURY, J. W. DRAPER.....Rochester, Minn.
MAYER, ALFRED.....Marine Laboratory of the Carnegie Institution, Tortugas, Fla.
MELTZER, S. J.....Rockefeller Institute for Medical Research.
MENDEL, LAFAYETTE B.....Yale University.
MEYER, ADOLPH.....Johns Hopkins University.
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MURPHY, JOHN B.....Northwestern University Medical School, Chicago.

NOGUCHI, HIDEYO.....Rockefeller Institute for Medical Research.
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OTT, ISAAC.....Medico-Chirurgical College, Philadelphia.
OTTENBURG, O.....Rockefeller Institute for Medical Research.

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SALANT, WILLIAM.....U. S. Department of Agriculture, Washington, D. C.
SCHULTZ, W. H.....U. S. Public Health and Marine-Hospital Service, Hygienic Laboratory, Washington, D. C.
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SENIOR, H. D.....New York University.
SHAFFER, PHILIP A.....Cornell University Medical College.
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SIMPSON, SUTHERLAND.....Cornell University, Ithaca, N. Y.
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STEINHARDT, EDNA.....Department of Health, New York City.
STEWART, GEORGE N.....Western Reserve University, Cleveland.
STEWART, HUGH A.....Columbia University.
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Teague, Oscar.....U. S. Department of the Interior, Bureau of Science, Manila.
Terry, B. T.....Rockefeller Institute for Medical Research.
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Weil, Richard.....Cornell University Medical College.
Welch, William H.....Johns Hopkins University.
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Wilson, Edmund B.....Columbia University.
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Wood, Francis C.....Columbia University.
Woodruff, Lorande Loss.....Yale University.

Yatsu, Naohidé.....University of Japan.
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Total number of members at the close of the academic year, 1910–'11: 222.

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INDEX

OF THE

SCIENTIFIC PROCEEDINGS.

[THE NUMERALS IN THIS INDEX CORRESPOND WITH THE NUMERALS IN PARENTHESIS ABOVE THE TITLES OF THE ABSTRACTS. PAGES ARE NOT INDICATED.]

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Wistar Institute of Anatomy (*Philadelphia*).—H. H. Donaldson, Shinkishi Hatai.
Barnard Skin and Cancer Hospital (*St. Louis*).—Leo Loeb.

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Auer, Benedict, Cole, Famulener, Field, Foster, Gies, Jackson, Joseph, Lambert, Levin, MacCallum, Mandel, Meltzer, Mendel, Morgan, Pappenheimer, Ringer, Rosenbloom, Rous, Sherman, Storey, Steinhardt, Swift, Terry, Wallace.

Members elected at the forty-fourth meeting:

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Date of the next regular meeting:
October 18, 1911.